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INSTITUTO DE MEDICINA TROPICAL

PROCEEDINGS OF THE SIXTH INTERNATIONAL CONGRESSES ON TROPICAL MEDICINE AND MALARIA

Lisbon, September 5-13, 1958

VOLUME II



LISBON, PORTUGAL

Anais do Instituto de Medicina Tropical
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DIVISION A

TROPICAL MEDICINE

SECTION 1
HELMINTHIC INFECTIONS

SUBSECTION 1
SCHISTOSOMIASIS

1ST SESSION: *Monday, September 8th — 9:00 to 10:30 a.m. Room D*

The meeting was opened by the Chairman, Dr. E. Carrol Faust, being present the Rapporteur, Dr. F. S. Barbosa, and the Secretary, Dr. M. Costa Faro. The following scientific papers were heard and the discussion was adjourned to the end of the 2nd session.

REPORT ON SCHISTOSOMIASIS

a) **BILHARZIASIS IN AFRICA AND EGYPT**

A. HALAWANI ¹

INTRODUCTION

The *Planorbidae* or the snails carrying bilharziasis (schistosomiasis) appeared in the Cainozoic age, while man has appeared at a later stage of evolution. However, the life cycle of *Bilharzia* worms might have been proceeding in his predecessors. The oldest proved bilharziasis case was detected by Ruffer (1910) in an Egyptian mummy of the twentieth dynasty (1200 B. C.). Bilharz was the first to discover the causative worm in Cairo in 1852. Meckel von Hemsbach, in 1856, named it *Bilharzia haematobia*; Weinland, in 1858, named it *Schistosoma haematobium*. Other species were described later.

¹ The Author did not attend the Congresses.

Human bilharziasis is widespread in the world, affecting about 150 million persons. The disease is widely prevalent in the continent of Africa. The routes of its spread are discussed.

The identification of the snails which transmit bilharziasis requires further work. The importance of the internal anatomy in classification requires evaluation. The study of the ecology of snails is still at a preliminary stage.

The control of the complications of bilharziasis depends mainly on mass-treatment of the population. The value of molluscicides is under debate by the experts working in different countries, because conditions and the habits of snails vary from one country to another.

In Egypt success has been achieved in certain limited areas by the application of copper sulphate or recently sodium pentachlorophenate. Taking the country as a whole, the treatment of the population and the partial use of copper sulphate as a molluscicide have reduced *S. mansoni* infection from 30 to 6 percent.

The relation of irrigation to the spread of schistosomiasis is discussed, in particular the effect of basin irrigation as compared to lift irrigation and perennial irrigation.

The clinical complications of bilharziasis, formerly frequent in Egypt, are at present rarely seen. Perennial fistulas have completely disappeared through spread of treatment. Polypi of the bladder, stones in the urinary system and hydro- and pyonephrosis have become less frequent. The writer has estimated that the occurrence of cancer of the bladder has been reduced to one fifth of its original incidence. Cytological diagnosis of cancer of the bladder by means of examination of the urinary sediment has been devised by the author as routine procedure in shedding tumours. Recent radiological methods, such as implantation of radioactive isotopes in cancers of the bladder, as well as the operation of the artificial bladder hold new hope for the bilharzial bladder cancer patient.

Cardio-pulmonary disease is the second severest complication of bilharziasis. Research on this complication is conducted in Egypt by various clinicians and cardiologists. In a radiological survey conducted among 5,042 individuals of the general population, cor-pulmonale with pulmonary aneurysmal dilatation was found to exist in 0.24 percent; cases of pulmonary artery enlargement of various grades was found in 2.45 percent.

Lieno-hepatic fibrosis of bilharzial origin is believed to be due to miracidial toxins excreted from eggs deposited in the liver. Studies on serum proteins in such conditions are discussed.

The treatment with Stibophen has given rise to fatal cases associated with haemoglobinurea in a certain number of them. The author has reported this condition. Investigations have shown that the kidney undergoes cortical degeneration, and suppression of urine takes place. The recovery of the patient depends on the response of the kidney.

Potassium antimony tartrate (tartar emetic) is replacing Stibophen, because it has been found to be far less associated with sudden death than Stibophen. If the latter gives 50 accidents per million, the accidents due to the former drug do not exceed 5 per million.

Lucanthone hydrochloride has been used extensively in Egypt. The author has finally found that a course of 10 mg/kg body weight daily for 20 days (i.e., 200 mg three times daily for an adult weighing 70 kg) is tolerated better than higher doses. Children below 10 years of age tolerate the drug better than adults. In bilharziasis haematobia the cure rate is approximately 70 percent, while in bilharziasis mansoni it is about 25-30 percent.

With regard to diagnostic methods of intestinal bilharziasis, the author has come to the conclusion that concentration methods of eggs in the stools are more reliable than rectal biopsy. Almost in all cases the eggs of *Schistosoma haematobia* present in rectal biopsy specimens are dead eggs and therefore give no true indication of active infection.

**b) SCHISTOSOMIASIS MANSONI IN THE AMERICAS;
SOME ASPECTS OF THE PROBLEM**

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Schistosoma mansoni is the only human species of schistosomes reported to occur endemically in the American continent, where it was probably introduced from Africa during the slave traffic.

The main endemic areas in the New World are situated in Puerto Rico, Venezuela and Brazil. Yet evaluation of the literature provides reliable data on the presence of the disease in Dutch Guiana (South America) and in the following West Indies islands: Dominican Republic, French St. Martin, St. Kitts, Antigua, Guadeloupe, Martinique, Sta. Lucia, St. Croix, St. Thomas and Vieques.

Small mammals (rodents and marsupials) were found naturally infected with *S. mansoni* in Brazil, but the epidemiological significance of these findings is not completely understood at the present moment.

The generic position of the snail vectors of *Schistosoma mansoni* is open to question. The species of the genus *Taphius* of the Titicaca Lake studied by Hubendick (1955) are not generically delimited neither from *Australorbis* nor from *Tropicorbis*. There are not also generic differences between the African genus *Biomphalaria* and the American genera *Tropicorbis* and *Australorbis*. Discussing these problems Barbosa (1956) suggested that the snails should be temporarily kept in the genera *Biomphalaria*, *Tropicorbis* and *Australorbis*. Hubendick (1957) believes that the genus *Biomphalaria* could include all the

African and American vectors of *S. mansoni*, admitting, notwithstanding, that this decision requires adjustments in the nomenclature. On the other hand, Paraense (1957) decided that the Brazilian species should be placed under the genus *Taphius*.

Barbosa (1956) discussed the problem of the discrimination of species in the planorbids. Although some of the species do not show complete reproductive isolation, temporarily 5 species can be maintained as such. They are: *Australorbis glabratus* (Say), *A. nigricans* (Spix), *A. incertus* (Lutz), *Tropicorbis centimetralis* (Lutz) and *T. janeirensis* (Clessin).

The three proved snail vectors of *S. mansoni* have large distribution over the American continent. The main and well known vector, *A. glabratus*, sometimes referred to as *A. olivaceus* and *A. gaudeloupensis*, occupies extensive territories in the West Indies and South America. *T. centimetralis* (= *T. stramineus*) is found from the Amazon region down to the central states of Brazil. *A. nigricans* (= *A. tenagophilus* or *A. immunis*) is found in central and south states of Brazil.

Two potential hosts of *S. mansoni* are known living out of the endemic areas. *Tropicorbis havanensis* from the southern states of the U.S. was proved susceptible to infection (Cram *et al.*, 1945) and Barbosa and Barbosa (1957) succeeded in infecting *T. chilensis* from Santiago (Chile) with a Brazilian strain of the trematode.

In large areas of Northeast Brazil the only vector is *Tropicorbis centimetralis*, which is not an efficient host of the trematode. Studies conducted with Southern Brazilian species revealed that some populations of *A. nigricans* could transmit the disease while others were completely resistant to the infection with *S. mansoni*.

Important experiments conducted by Files (1951) revealed that the trematode as well as the snail hosts showed physiological differences between continents.

A. glabratus is the main vector of the trematode in Brazil as well as in other American countries. In spite of that, the species is not uniformly susceptible and the strain from the state of Bahia (Brazil) was proved to be not only refractory to the exotic strains of the parasite as demonstrated by Files, but also partially resistant to the infection with the Brazilian strain of *S. mansoni*.

Comparatively little is known about the ecology of the snail vectors in the Americas, notwithstanding recent studies conducted in Northeast Brazil and Puerto Rico. The distribution and habits of the snails, their life cycles, their resistance to physical and chemical agents, the physiography of fresh-water habitats and the ecology of the larval stages, are the most important problems recently investigated (Olivier and Barbosa, 1955, *a* and *b*, Barbosa and Coelho, 1955, Harry and Cumbie, 1956, *a* and *b*).

There is evidence that two ecological races of *A. glabratus* exist in Brazil in regard to their capacity to resist desiccation. It is worth-while to mention the recent paper of Newton and von Brand (1955) where these workers definitely demonstrated that two strains of *A. glabratus*, one from Venezuela and other from Brazil, differ physiologically.

The common belief that snails harboring schistosomes larvae were unable to survive desiccation was shown to be incorrect by Barbosa and Coelho (1955) and no methods could ever be applied to control the disease on the acceptance of this fact. It would be desirable to know if, in other endemic areas, *S. mansoni* sporocysts are able to live in diapause when the snails are out of water, as it was proved to be true with the Brazilian strain of the trematode.

The hepatic lesions in schistosomiasis mansoni in Brazil was recognized to be the same described by Simmer in Egypt as «clay-pipe-stem-cirrhosis». Nevertheless discrepancies exist concerning the factors responsible for the production of the anatomical liver picture. According to Bogliolo (1957), the eggs carried through the small nondichotomic portal branches act direct or indirectly producing chronic fibrosing granulomatous periphlebitis and the lesions are limited to the connective periportal sheath. According to other authors (Barros Coelho, 1950, Magalhães Filho, 1952), dead worms are the most important pathogenic agents whose desintegrated bodies would cause trombosis of the intrahepatic portal vascular branches with necrosis and destruction of the local portal spaces.

Recent studies on the experimental pathology of schistosomiasis mansoni have been very fruitful. Warren (personal communication) was able to produce portal hypertension and esophagal varices in infected mice and Magalhães Filho (1957) noticed that the spleen

of mice attained enormous size in the re-infected group of animals. Pulmonary lesions produced in resistant mice after a challenger exposure of cercariae of *S. mansoni* has been studied by the last worker.

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ON THE FAMILY ANCYLIDAE WITH SPECIAL
REGARD TO *FERRISSIA TENUIS* (BOURGUIGNAT),
THE SUSPECTED INTERMEDIATE HOST OF *SCHIS-*
TOSOMA HAEMATOBIIUM IN INDIA

BENGT HUBENDICK

Göteborg

In 1955 Cadgil and Shah stated that *Schistosoma haematobium* was transmitted by the ancyloid snail *Ferrissia tenuis* (Bourguignat) in the Bombay area. This is an unexpected statement. It seems *a priori* much more likely that *Indoplanorbis exustus* (Deshayes), also present in the same area, would provide an intermediate host as it is a planorbid of the same subfamily as the African vectors of *Shistosoma haematobium*. Anyhow, a certain interest has now focused on *Ferrissia tenuis* and a few facts about this snail may be worth-while here.

The *Ancylidae*, or the fresh-water limpets, constitute a family with world-wide distribution. Its members are small and have a cap or shield shaped shell. Their visceral hump is more or less pyramidal, not spiral, though a slight asymmetry of the shell indicates a vestigial spiral coiling. The mantle cavity is partly or almost entirely reduced.

There is a considerable anatomical variation within the family. Two subfamilies are generally distinguished. *Acroloxinae* has an organization corresponding to a dextral torsion. There the mantle cavity, the female and male pores, the renal and anal pores, and the pseudobranch are situated on the right side of the body. Apart from one widely distributed species there are a few restricted to the Bajkal Lake, in Siberia, and the Ochrid Lake, in Yugoslavia. One additional species is known from Colorado, in U.S.A. All other *Ancylidae* are considered to belong to the subfamily *Ancylinae*. In this subfamily the anatomy corresponds to a sinistral torsion. All the openings men-

tioned above and the pseudobranch are situated on the left side of the animal.

Ancylinae comprises several genera. The validity and distinction of these are not satisfactorily clarified. The author is in the course of revising the taxonomy of the family. So far not even the validity of *Ferrissia* is definitely settled. There are certain important anatomical differences between *Ferrissia tenuis* (Bourguignat) and the type species of *Ferrissia*. Therefore the final systematical position of *F. tenuis* is still unknown.

The shell reaches about 3 mm to 3,5 mm in length and about 2 mm in breadth. The relative height varies considerably, but is normally between one half and one third of the breadth. The longitudinal edges are largely parallel. The anterior margin is often more bluntly rounded than the posterior. The apex of the shell is located not far from the posterior margin and slightly to the right of the median line. An extremely fine concentric growth sculpture and a still finer radial sculpture are present. The yellowish or brownish periostracum extends outside the calcareous shell border. This is the rule in *Ancylidae* and allows the animals to press their shells tightly towards a level substratum. The shell of *F. tenuis* exhibits a considerable eco-phenotypical variability.

Under the shell margin there is an eaves-like mantle border all around the animal. The tentacles are comparatively short and bluntly tapering. At the base of either tentacle and behind the eyes there is a projection with sensory epithelium. The foot has a somewhat triangular sole. Below the posterior part of the mantle cavity there is a simple, unfolded projecting flap, the pseudobranch. The rectum passes through this flap and the anal pore is located on it. In other *Ancylinae* the pseudobranch can be strongly folded, or consist of two folded flaps.

Three strong shell adductors are branching off from the foot musculature, one anteriorly on either side and one posteriorly on the left side. This muscular system is more or less continuous and horseshoe-shaped in some forms. Near the left anterior muscle is an osphradium in the underside of the mantle border. Behind the same muscle is the mantle cavity, which is comparatively large in *F. tenuis*.

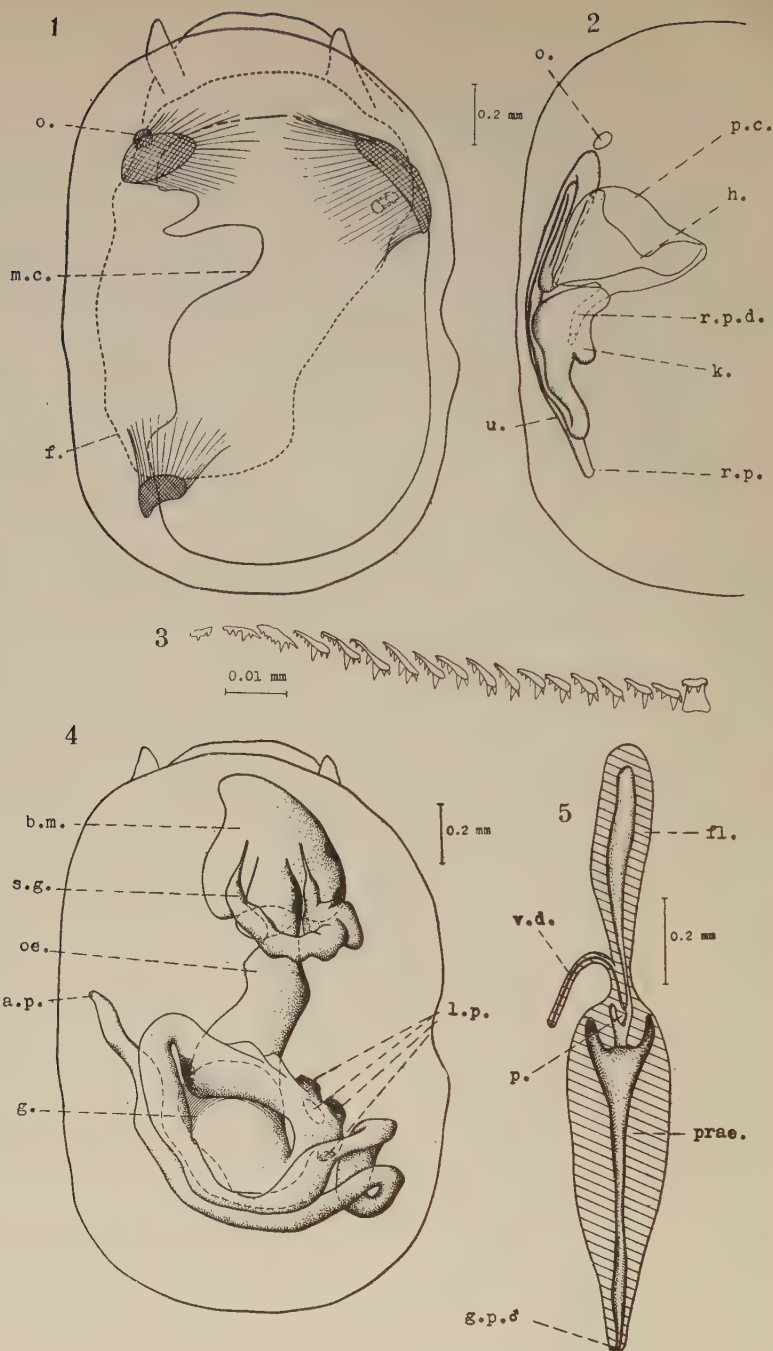
The rather large pericardium is located dorsally of the mantle cavity. From the ventroposterior border of the former the long, ciliated reno-pericardial duct goes out and enters the proximal chamber of the kidney. The latter continues with a slender duct in the left mantle border. The narrow efferent renal pore is situated far posteriorly on the inside of the mantle border.

The circulatory system has not been examined in *F. tenuis* though in *Acroloxus lacustris* Müller. Walled arteries lead the blood from the heart to two large haemocoels, one around the buccal mass and another around most of the viscera. The former has almost complete walls, the latter very incomplete and empties into lacunae between various tissues but without walls of their own. The venose blood goes to lacunae in the mantle border and then a part of the blood passes through the gill, and another part vascularizes the kidney before reaching the atrium of the heart. At a temperature of 25°C there are about two heart beats per second in an 1 mm long individual of *F. tenuis*.

The alimentary system has a complicated buccal mass, two salivary glands which are connected behind, a wide oesophagus, a gizzard, a liver, and a coiling intestine. In the region where the pylorus merges into the intestine there are four pores from the liver. In other species there are generally fewer liver pores. The jaw consists of numerous minute chitinous scales. The radula of a full-grown specimen has about 35 teeth in a queer row. The centrals and the inner laterals are fourcuspid. The number of cusps increases laterally but decreases again in the marginals. At 25°C an 1 mm long specimen of *F. tenuis* accomplishes about one movement cycle of the buccal system per second.

The central nervous system has not yet been examined in *F. tenuis*. In ancyliids where this structure has been examined it is extremely concentrated. The pleural ganglia can be fused with the parietal ganglia, leaving only one independent visceral ganglion. Even the connectives between the abdominal and the pleuroparietal ganglia can contain nerve cells. Lever (1957) has stated the presence of neurosecretion in *Ferrissia* sp.

The reproductive system consists of an hermaphrodite gland and duct. The latter divides up into the female and male duct in the carrefour, where also the albumen gland connects. This gland can



Figs. 1-5 — *Ferrissia tenuis* (Bourg.) — 1. Schematic diagram showing the shell adductors: *f.*, contour of foot; *m.c.*, line indicating bottom of mantle cavity (continues anteriorly and posteriorly as the inner margin of the mantle border); *o.*, osphradium. 2. Excretory system: *h.*, heart; *k.*, kidney; *o.*, osphradium; *p.c.*, pericardium; *r.p.*, renal pore; *r.p.d.*, reno-pericardial duct; *u.*, urethra. 3. Half a cross row of the radula. 4. Alimentary system: *a.p.*, anal pore; *b.m.*, buccal mass; *g.*, gizzard; *l.p.*, liver pores; *oe.*, oesophagus; *s.g.*, salivary gland. 5. Male copulatory organ: *fl.*, flagellum; *g.p.♂*, male genital pore; *p.*, penis; *prae.*, praeputium; *v.d.*, vas deferens

sometimes be tremendously hypertrophic. The female duct with its glands form a rather closely packed unit where also the prostate, when present, is entangled. The spermatheca has a slender duct. The male copulatory organ has a well-developed praeputium, a small penis with a lateral pore, and a glandular flagellum. The organization of the reproductive system shows a considerable variability in *Ancylidae*.

F. tenuis is often aphillic, i.e., the male copulatory organ is lacking. In aphillic specimens the seminal vesicle, the prostate and the vas deferens can be partly or totally lacking. In one examined aphillic specimen spermatozoa did not occur in the gonad.

Aphilly seems to be common in *Ancylidae*. Of 10 examined specimens of *Ancylus sharpi* Sykes from Hawaii all were aphillic and without spermatozoa in the whole system. Parthenogenetic reproduction might occur. In *A. australicus* Tate 168 of 178 examined specimens were aphillic. In one of a few sectioned specimens there were spermatozoa in the thick, blind-ending vas deferens.

The ancylid species live either in running or in stagnant water, or in both. They move about slowly on their substratum, which is generally stones, submerged vegetation, or dead vegetable matter. Thanks to their small size and their form, which give a proportionately large free body surface, they do not need air breathing. Their shallow mantle cavity would hardly permit that type of breathing.

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SURVIVAL AND WEIGHT LOSS OF *AUSTRALORBIS*
GLABRATUS KEPT AT CONTROLLED RELATIVE
HUMIDITIES IN THE LABORATORY

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Barbosa and Dobbin (1952) and Olivier and Barbosa (1955a, 1955b) have shown that the two vectors of schistosomiasis in North-eastern Brazil, *Australorbis glabratus* and *Tropicorbis centimetralis*, can survive in natural habitats that are without standing water for many months. These field studies indicated that the surviving snails pass the dry season at or near the soil surface in the shade and under protection of vegetation and debris. When the end of the wet season comes and the pools and marshes dry, the planorbid vectors die unless they are located where the environment is especially favorable for survival out of water. The exact nature of the conditions favoring the survival of the snails are not known, but it is reasonable to surmise that high humidity, shade, and concealment would protect the snails

¹ Laboratory of Parasitic Diseases.

against excessive water loss, against heat death, and against animals which might eat them.

When the snails were taken into the laboratory and kept out of water in clay jars or in wooden boxes on soil where the relative humidity was fairly high and the temperature was moderate, some of the snails lived for a very long time (Olivier, 1956*b*, Olivier and Barbosa, 1956).

Although both the field and laboratory observations indicated that the snails probably survive best when the relative humidity is high, they gave no precise information as to what the humidity tolerance limits of the snails are or how much weight loss the snails will withstand. It was important to obtain accurate information as to how the Brazilian vectors of schistosomiasis respond to known relative humidities. Since measurement of the relative humidity in microenvironments in the field is difficult and equipment for doing this was not available when extensive field studies were being made in 1953 and 1954, a series of controlled laboratory experiments were conducted instead. Observations were made on the survival time and the pattern of weight loss of *A. glabratus* when the snails were held at various relative humidities in closed jars. The results of some of these experiments are presented here. [Some of the data have been presented briefly in a previous report (Olivier and Barbosa, 1954).]

MATERIALS AND METHODS

All the snails were collected from the field within a few days of the start of the experiments. Most of the snails were collected from habitats that had been well studied and for which field conditions and field survival characteristics of the snails were known.

After collection, the snails were brought into the laboratory where they usually were kept for several days before the experiment was begun. Snails collected dry were used as they came from the field. Snails collected from the water were isolated in water for 24 hours to test whether they had mature trematode infections. Snails found to be infected were not used. Except for this one day in water, they were always kept out of water in moist, unglazed clay jars until the experiment was begun. There is a possibility that some of the snails

collected dry from the field may have been infected with *Schistosoma mansoni* or other trematodes. This is considered highly unlikely, however, since it was determined by examination of snails from the same collections that trematode infections were very rare in these snails.

Snails to be used in the experiments were wiped to remove dirt and moisture before their initial weighing. They were then fastened to numbered paper strips by means of transparent cellulose tape so that each snail could be identified. During the experiments the paper strips with their attached snails were suspended in large-mouthed glass jars of about 2 l capacity. The glass lid was ground to fit and sealed with paraffin. From time to time the snails were removed from the jars for inspection and weighing.

Relative humidities ranging from 96 to 0.5 percent were created in the jars. The humidities used and the substances used to create them are shown in the table below. In some of the experiments snails

Relative humidities used and substances producing them

Relative humidity (percent)		Substance producing the stated relative humidity
Average	Range	
96	95-97	Water
92	—	Saturated solution of K_2SO_4
85	83-87	Saturated solution of $ZnSO_4$
74	72-76	Saturated solution of $NaCl$
57	—	Saturated solution of $NaBr$
30	28-31	Saturated solution of $CaCl_2$
0.5	—	Dry $CaSO_4$ or $CaCl_2$

were also held in open moist clay jars in which the relative humidity was estimated to range from 90 to 95 percent. The relative humidities actually achieved in the jars were not determined because no suitable equipment was available. The stated humidity levels are those obtained in desiccators in this laboratory using an Aminco electric hygrometer (von Brand *et al.*, 1957), except for those of K_2SO_4 and dry $CaSO_4$ and $CaCl_2$, which were taken from Ludwig (1937).

All the experiments were conducted at laboratory temperatures which ranged from 25°C to 29°C. The snails were weighed, to the nearest milligram, usually every 3 or 4 days. At each weighing the snails were examined for viability by passing a strong beam of light through the shell in a manner similar to that used in candling eggs. This made it possible to detect dead snails with a high degree of accuracy. Normally, as the snails dry, they gradually retract into the shell. In a living, retracted snail the part of the shell occupied by the snail's soft parts is uniformly opaque and the free boundary of the foot makes a sharp, clear line readily seen through the shell in transmitted light¹. If the snail is dead, the free margin of the foot is usually not sharp and regular and the part occupied by the soft parts is not uniformly opaque because of shrinkage away from the shell or the formation of gas or fluid within the body after death. When there was doubt as to the viability of a snail, it was removed from the experiment and tested in water. Whenever possible, snails surviving to the end of an experiment were tested for viability by putting them in water for 24 hours. Unless it is otherwise noted, the weight data include only the weights of the snails surviving to the last date of record and tested for viability by water immersion.

EXPERIMENTS

Experiment 1

The snails for this experiment were collected from a small stream in Pernambuco which is subject to great fluctuations in water level but never dries completely. In this habitat, some of the snails may be stranded out of water during the dry season, but there is no time when snails cannot be found in the stream. After collection on June 10, 1953, the snails were held in a shallow clay jar for one

¹ A few specimens of *A. glabratus*, on drying, retained a large air bubble in the respiratory chamber and this could be seen in transmitted light to occupy one-third to one-half of the terminal whorl of the shell behind the foot. These snails never retracted more than about one-third of a whorl but they survived about as well as the snails which retracted typically.

month at a relative humidity (r.h.) of about 90-95 percent. During this time there were very few deaths. Eighty of the survivors were then divided into 4 groups of 20 each. They averaged 0.481 g in weight and 17.0 mm in greatest diameter (range 13-22 mm). Group I was returned to the clay jar and the other 3 groups were put at 85, 74, and 57 percent r.h.

The snails lost weight (Fig. 1) during the next 42 days in amounts that were apparently dependent upon the humidity of the

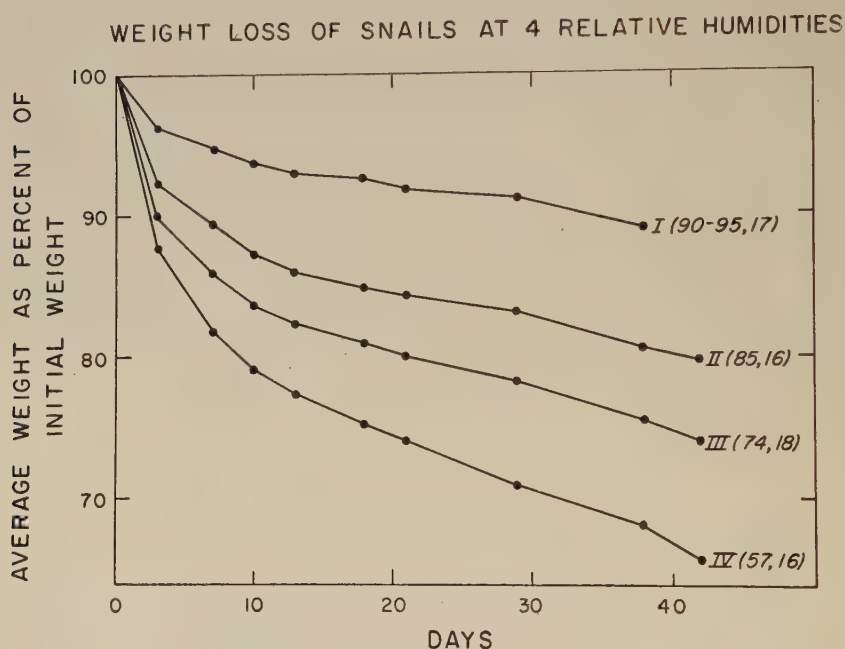


Fig. 1 — Weight loss of snails in experiment 1. The curves for the groups of snails are indicated by Roman numerals. The relative humidity and the number of snails providing the data for the curve are given in parentheses after the numeral

atmosphere in which they were kept. The flatness of the curves may be due, in part at least, to the fact that snails were held out of water for a month before they were weighed for the first time and so were partly desiccated before the experiment was started.

Death losses also were related to the humidity at which the snails were kept. After 42 days the numbers of surviving snails in

the 4 groups (I to IV) were 16, 16, 18, and 16. After 54 days, the numbers were 16, 15, 11, and 7.

Apparently the degree of retraction of the snails into the shell is directly related to the degree of desiccation. At the start of the experiment, the snails were all retracted about the same amount and the average retraction was 0.33 whorl. On the thirty-third day the average retractions, in fractions of a whorl, were: Group I—0.54; Group II—0.70; Group III—0.88; and Group IV—0.92.

Experiment 2

On July 9, 1953, 80 snails were collected from a pool in Pernambuco which contains water only during the wet season, which lasts from 5 to 7 months. The snails were held out of water in moist unglazed jars at a relative humidity of about 90-95 percent for one day and were then weighed and measured. They averaged 24.5 mm in greatest diameter (range 20-29 mm) and averaged 1.460 g in weight. They were divided into 4 equal groups and held at different humidities as follows: Group I—85 percent; Group II—74 percent; Group III—30 percent; Group IV—0.5 percent.

As would be expected, the greatest weight losses (Fig. 2) were in the snails at the lower humidities. Severe death losses occurred only among the snails at the lower humidities. After 28 days the numbers of surviving snails in the 4 groups (I to IV) were 18, 20, 20, and 16. After 47 days the numbers of survivors were 18, 18, 8, and 3. Sixteen snails of Group I and 15 of Group II survived until the experiment was stopped at 71 days.

Experiment 3

On July 24, 1953, 80 snails were collected from the same locality which provided the snails for experiment 1. The snails were held out of water in a moist clay jar for 3 days and then measured and weighed. Their average diameter was 24.5 mm (range 15-25 mm) and their average weight was 1.460 g. They were divided into 4 equal groups and placed at the following relative humidities: Group I—

90-95 percent (in an uncovered, unglazed clay jar); Group II — 85 percent; Group III — 57 percent; and Group IV — 0.5 percent.

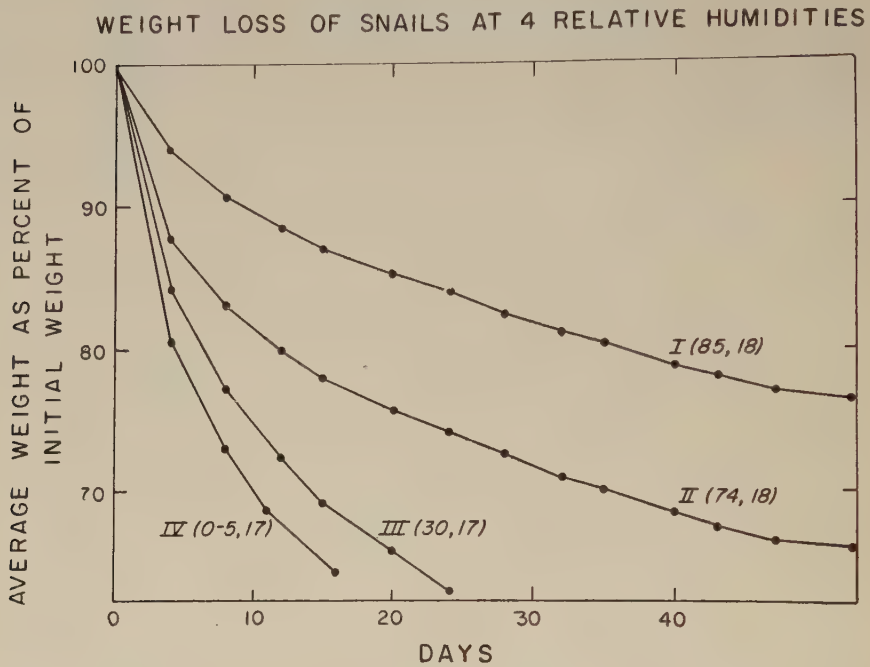


Fig. 2 — Weight loss of snails in experiment 2. The curves for the groups of snails are indicated by Roman numerals. The relative humidity and the number of snails providing the data for the curve are given in parentheses after the numeral

Again, weight and death losses were related to the relative humidity at which the snails were held (Fig. 3). Within the short span of the experiment, the snails at the high relative humidities lost very little weight, but the weight of the snails at 0.5 percent r.h. fell rapidly. Death losses were infrequent at first. After 25 days the numbers of surviving snails in the 4 groups (I to IV) were 16, 16, 15, and 18. However, at the lowest humidity many deaths soon followed and after 38 days the numbers were 16, 15, 14, and 2. Fourteen days from the start of the experiment the average retractions of the snails, in fractions of a whorl, were: Group I — 0.56; Group II — 0.72; Group III — 0.68; and Group IV — 0.86.

The following 3 experiments were done with snails collected during the wet season from a habitat in Pernambuco that is dry for 5 to 7 months every year (Pool 1 in Olivier and Barbosa, 1955a). In 2 of the experiments the snails were compared with *A. glabratus* collected from a lake in Salvador, Bahia. The level of the lake is relatively constant throughout the year and presumably the snails in it are not subject to desiccation.

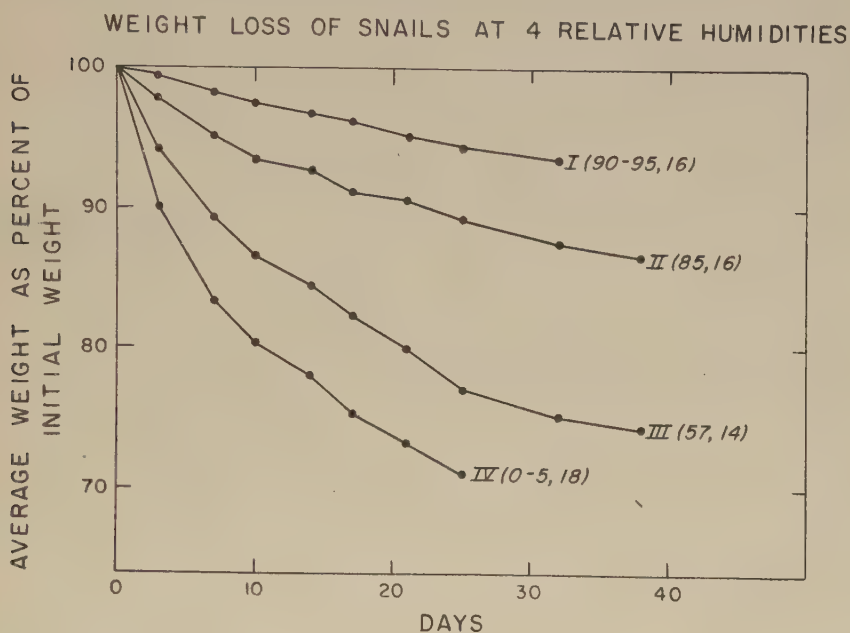


Fig. 3 — Weight loss of snails in experiment 3. The curves for the groups of snails are indicated by Roman numerals. The relative humidity and the number of snails providing the data for the curve are given in parentheses after the numeral

Experiment 4

Ninety snails were collected from the temporary pool on August 24, 1953. They averaged 27.0 mm in diameter (range 25-29 mm). The snails were held at 90-95 percent r.h. for one day and then were weighed and divided into 4 groups. At this time their average weight was 1.870 g. They were then dispersed into jars at 4 relative humidities: 96, 85, 74, and 57 percent.

The pattern of weight loss for the snails in this experiment was similar to that of the previous experiments (Fig. 4). At the start of the experiment the numbers of snails in the 4 groups (I to IV) were 40, 20, 20, and 10. After 30 days the numbers were 36, 19, 14, and 9. After 41 days the numbers were 36, 18, 12, and 6.

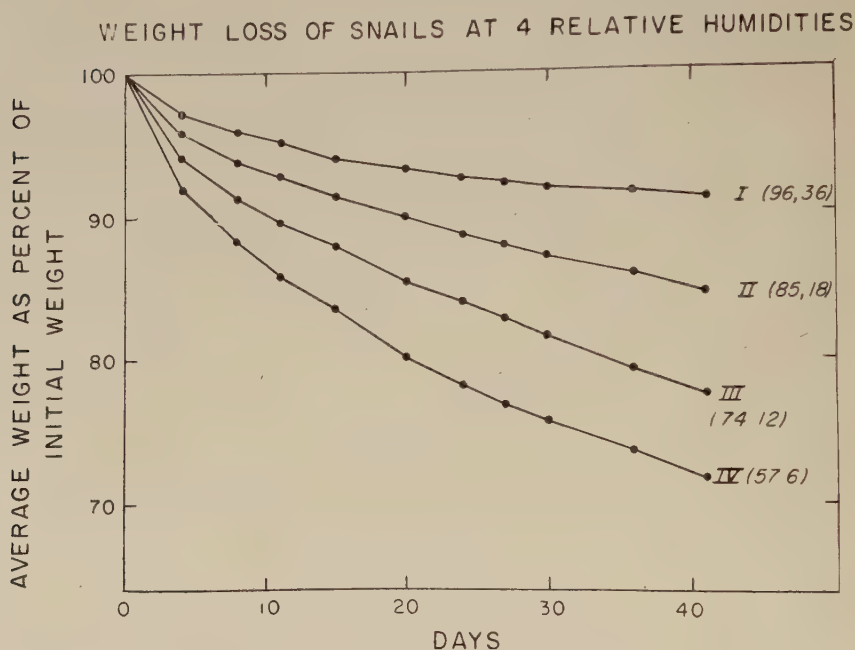


Fig. 4 — Weight loss of snails in experiment 4. The curves for the groups of snails are indicated by Roman numerals. The relative humidity and the number of snails providing the data for the curves are given in parentheses after the numeral

Experiment 5

In this experiment, and in the next one, snails from the temporary pool were compared with snails of approximately the same weight and diameter from a permanent lake, the Dique de Tororó, in Salvador, Bahia. It was known that snails from the temporary pool in Pernambuco could survive out of water for long periods in the field. The response of the Bahia snails to desiccation was unknown. However, it was postulated that since the Bahia snails lived in a

permanent body of water and were not subject to great water level fluctuations, they might not be capable of long survival out of water.

On October 1, 1953, snails were collected from the lake in Salvador and on the same day they were flown to Recife, packed in moist leaves. On arrival, less than 6 hours after they were collected, they were placed in water and kept there until used. Very few snails died as a result of this treatment. The snails from the pool in Pernambuco were collected on October 5. On October 6, 60 snails from each locality were divided into 6 equal groups. The Pernambuco

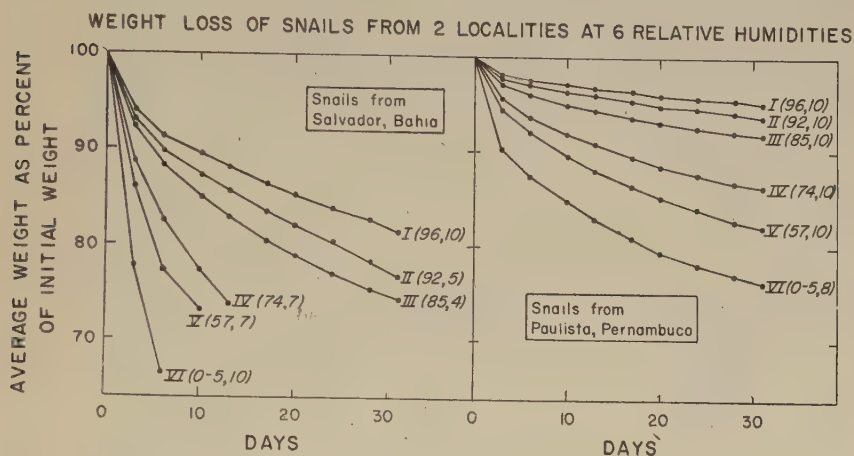


Fig. 5 — Weight loss of snails in experiment 5. The curves for the groups of snails are indicated by Roman numerals. The relative humidity and the number of snails providing the data for the curve are given in parentheses after the numeral

snails averaged 21.6 mm in diameter (range 18-25 mm) and averaged 1.099 g in weight (range 0.645-1.576 g). The Salvador snails averaged 21.1 mm in diameter (range 18-26 mm) and their average weight was 1.009 g (range 0.684-1.692 g). One group from each locality was placed at each of the following relative humidities: 96, 92, 85, 74, 57, and 0-5 percent.

After 30 days all surviving snails were placed in jars at 96 percent r.h. and on the 38th day all were tested for viability in water. Figure 5 demonstrates that the Salvador snails lost weight much more rapidly than did the Pernambuco snails at all humidities. They also died more rapidly. Of the snails at the 3 lowest humidities all died

within 24 days. Half of the snails at the 3 highest humidities died before the 38th day. On the other hand, almost all the snails from Pernambuco, treated identically, survived.

Experiment 6

This experiment is very similar to the preceding one. The snails were from the same collections and the experiment was run in the

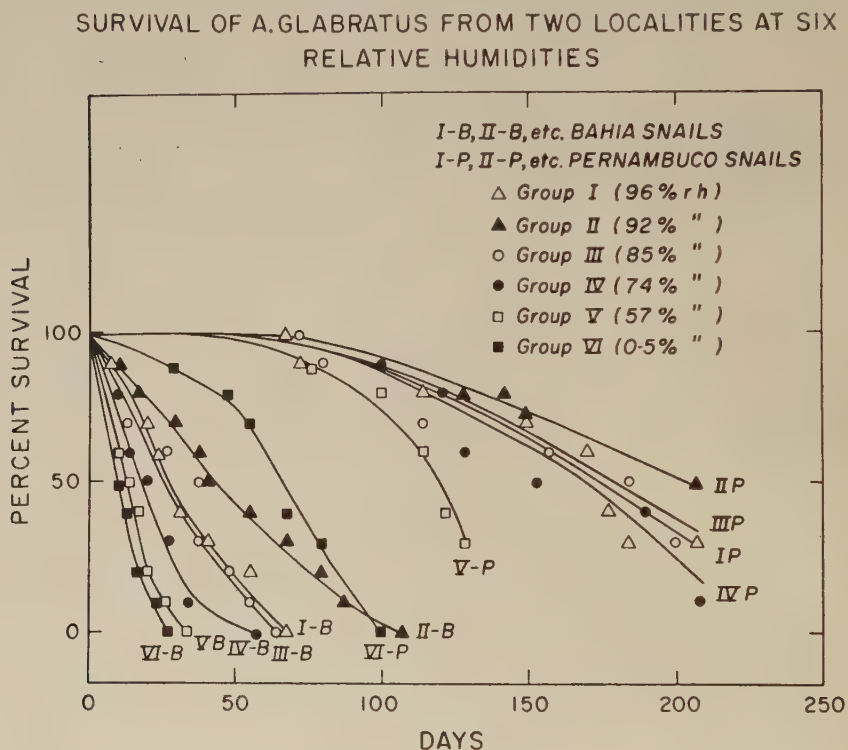


Fig. 6 — Survival patterns of the snails in experiment 6

same containers used for experiment 5. The experiment was started one day later and was continued longer.

The Pernambuco snails averaged 20.7 mm in diameter (range 16-26 mm) and their weights averaged 1.002 g (range 0.467-2.05 g). The Salvador snails averaged 20.7 mm in diameter (range 18-24 mm) and their weights averaged 0.972 g (range 0.653-1.364 g).

The snails from Bahia died very rapidly (Fig. 6), while the snails from Pernambuco survived much longer. The weight losses of the snails from the two localities also were very different (Fig. 7). The Bahia snails in all the groups lost weight rapidly, while at the higher humidities, at least, the Pernambuco snails lost weight very slowly.

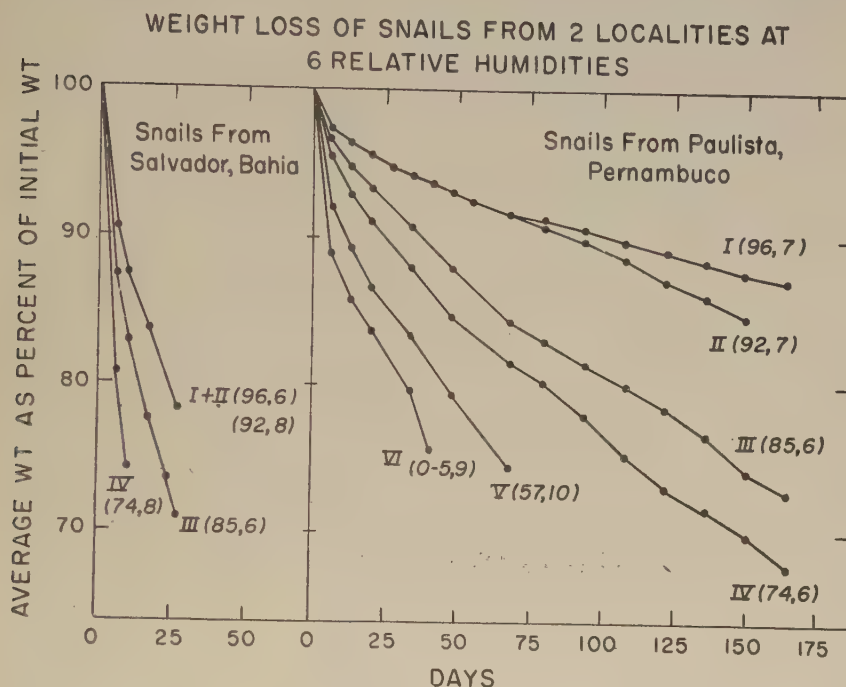


Fig. 7 — Weight loss of snails in experiment 6. The curves for the groups of snails are indicated by Roman numerals. The relative humidity and the number of snails providing the data for the curve are given in parentheses after the numeral

DISCUSSION AND CONCLUSIONS

In the first 4 experiments, done with snails from 3 localities near Recife, in Pernambuco, there were detectable, but not large, differences in drought resistance among the snail strains. The snails from all 3 habitats were able to survive well out of water when the relative humidity was high and even those at very low relative humidity lived

for extended periods. Not more than one-third of the snails was lost during the first 30 to 40 days from any of the groups held at 75 percent relative humidity and above. Generally, at relative humidities below 75 percent, the death losses were small during the first 4 or 5 weeks, but after that time the death rate accelerated greatly. Weight losses in all the experiments were very slow and gradual at the higher relative humidities. Weight losses among the snails at the lower relative humidities were greater, of course, but in no case were they precipitous.

Since the survival and weight loss records for the snails from Pernambuco agreed so closely in the 4 experiments, it was concluded that the snails of many, if not all, of the habitats in Pernambuco might be able to survive long periods out of water at least at the higher relative humidities. It then seemed desirable to compare these snails with others of the same species living in another geographic area and not subject to the same seasonal climatic changes. Snails from a lake in Salvador, Bahia, were selected for this comparison since the place is far from Pernambuco, assuring geographic separation; the snails were numerous; the water level of the lake is relatively constant; and there is a less marked seasonal rainfall cycle there.

The last two experiments compare snails from Pernambuco with those of the lake in Salvador and demonstrate that there is a striking difference in drought-resistance of the snails from the two localities. The Bahia snails were unable to live out of water at low relative humidities even for a short period of time. This is shown best in experiment 5, in which most of the snails at 0.5 percent relative humidity were dead within 10 days and at 31 percent r.h. only 2 of 10 snails survived 17 days. At the higher humidities there was longer survival, but the weight losses were fairly precipitous when compared with those of the snails from Pernambuco. The Bahia snails were not capable of long survival even in a water-saturated atmosphere and they must be considered highly drought-susceptible. Indeed, since the snails lost weight and died so rapidly at the lower humidities, it is possible that a majority of the snails became moribund soon after removal from the water.

It is interesting to compare the data from the Bahia snails with those presented in 1957 by von Brand *et al.* for a selected strain of laboratory-reared *A. glabratus* derived originally from Venezuelan stock. Their weight and death losses were fairly similar to those of

the Bahia strain. At the lower humidities the 50 percent death points (Fig. 8) for the Bahia snails fall somewhat later than those of the laboratory strain, indicating that, if anything, the Bahia snails were somewhat more drought-resistant than the laboratory strain.

The Pernambuco snails stand in striking contrast to the Bahia snails. They survived very long periods out of water at the higher

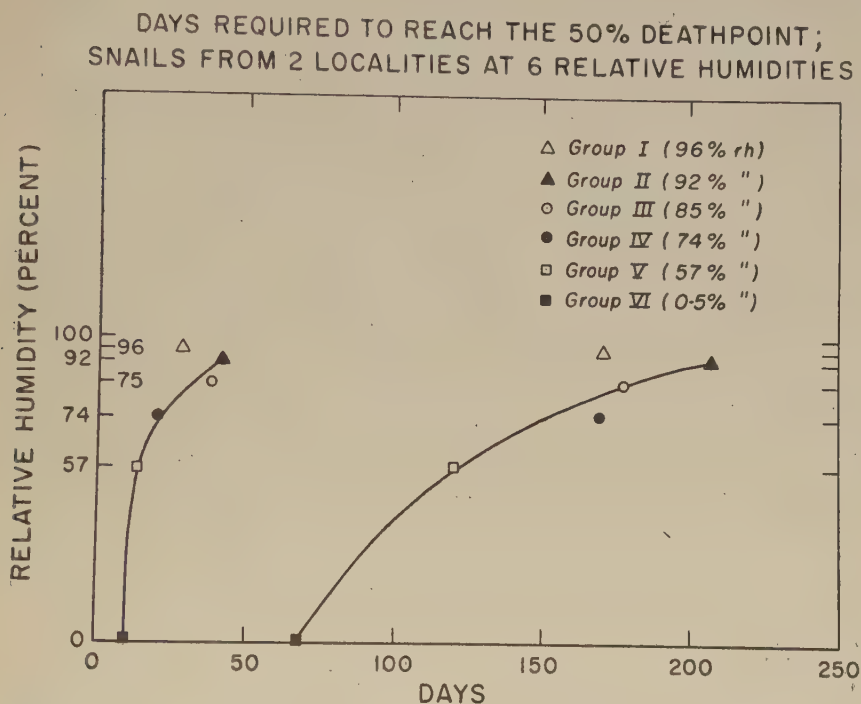


Fig. 8 — Fifty percent death points for the snails of experiment 6. The curve for the Bahia snails is at the left, while that for the Pernambuco snails is at the right

humidities and those at 0.5 percent r.h. sustained smaller death and weight losses than the Bahia snails, kept in a water-saturated atmosphere. Survival at the higher relative humidities was not surprising since it had been shown in field studies (Olivier, 1956; Olivier and Barbosa, 1955a, 1955b) that they could survive 5 to 7 months out of water in the protection of vegetation and debris. Their tolerance for extremely low humidity was surprising and impressive. In one

experiment, 3 of 10 snails lived 129 days at 31 percent r.h., at which time they were placed in water and so removed from the experiment. In the same experiment, half of the snails at 0.5 percent r.h. lived over 60 days. Although this indicates a high degree of drought-resistance in the Pernambuco snails, it is doubtful that the snails could tolerate such low relative humidities as well under field conditions since the environment is less stable than in the laboratory.

It should be pointed out that *A. glabratus* does not produce a true epiphragm and that none of the snails in these experiments even produced the thin, membranous films sometimes found in the shell opening of snails desiccating under field conditions. Thus, these snails had no mechanical protection against water loss.

The snails gradually retracted into their shells as they lost weight. At high relative humidities there is comparatively little retraction, at least during the first month, in the case of drought-resistant snails. At low relative humidities retraction is much faster. In either case retraction is correlated with the amount of weight lost and if the desiccation characteristic of the snails is known, the degree of retraction can indicate the approximate time the snail has been desiccating at a given humidity. The snails usually do not survive retraction of more than one whorl, though, in a few instances, snails retracted 1.25 whorls were found to be alive.

SUMMARY

Field collected *A. glabratus* were held out of water in the laboratory for an extended period at relative humidities ranging from 0.5 to 96 percent. Weight loss and survival records are presented for snails from three habitats in Pernambuco, Brazil, and from one in Salvador, Bahia. The Pernambuco snails survived for long periods out of water even at low relative humidities. On the other hand, the Bahia snails lost weight and died much more rapidly under identical conditions.

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SOME NEW APPROACHES IN MALACOLOGY

C. A. WRIGHT

In explaining the ecological concept of parasitism Baer (1952) stressed the importance of a sound taxonomic basis for work on host-parasite relationships. Recently, Mattingly (1957) has modified Ross' famous dictum thus: «In order to control malaria we must learn to think not like a mosquito but like a population of mosquitoes.» The medical malacologist must be even more adaptable, for not only has he got to learn to think like a population of snails, but he must try also to see those snails through the eye-spots of populations of miracidia. It is with these principles in mind that this paper is presented.

An ecological approach to the problem of schistosomiasis must involve consideration of the ecology of both the definitive and intermediate hosts, also of both the free-living and parasitic stages of the parasite. These aspects cannot be separated from one another, but for practical purposes they must be treated in parts. The concern of the malacologist is not only with the snail hosts, but also with the free-swimming miracidia and developing sporocysts within the snails. To a lesser extent he must also consider those aspects of human and molluscan ecology which result in the miracidia being brought into contact with the snails.

In an earlier publication (Wright, 1956) a summary of some of the factors affecting the susceptibility of snails to infection with schistosome miracidia was given. In this summary it was shown that chemotaxis probably plays an important part in the selection by a miracidium of a potential intermediate host. Recent work (Wright, 1959) on the lymnaeidae has shown that by the use of paper-partition chromatography species-specific substances can be identified in the body-surface mucus of snails. The specific compounds that

have been observed and which can be used for discrimination between species are not necessarily the same as those which might be detected by a miracidium and, until it is possible to identify the compounds to whose stimuli the larvae react, the results of this type of work must be interpreted with great caution. The discovery may have significance in another respect. Snails are largely non-visual animals and probably depend upon chemotactic recognition of one another. The specific substances in the mucus may play a part in this recognition and may be partly acting as an isolating mechanism preventing mating between closely related species living together in the same habitat.

The results obtained from the chromatographic studies on *Lymnaea* are interesting since they provide some indication of the problems which may arise in the application of this technique to other groups. Only the ultra-violet fluorescent patterns were studied in detail for, so far, it is only these which show easily the differences which are sought. Three main groups of results have been obtained, each presenting different problems in interpretation. The first group gives scarcely any fluorescent pattern (*L. truncatula*, *L. stagnalis* and *L. glabra*) and differences between their chromatograms can only be detected with the use of various reagents; these species are in no way closely related to one another. The second group (*L. peregra*, *L. auricularia* s.l. and *L. natalensis*) all give basically the same pattern of four blue fluorescent bands of varying intensities. Species differences occur in the relative intensities of bands or in their total absence; thus the outermost band in *L. peregra* is always brighter than the penultimate, but in *L. auricularia* these two bands are of roughly equal intensity. In *L. natalensis* the outermost band of the basic pattern is missing and the penultimate band is very bright. The third set of results have been got from one species only, *L. palustris*. Here the basic pattern consists of a double lilac-coloured band. In some populations only a single band can be detected and in others the weaker of the bands is barely visible. With the butyl alcohol-acetic acid-water solvent used throughout the work on *Lymnaea* these two bands are so close together that they almost merge, but if a propanol-ammonia solvent is used they can be clearly separated and their relative intensities more easily assessed. In addition to these population differences a seasonal variation has been detected. In the autumn a strong yellow band appears at a fairly high Rf value.

and the very light blue which is usually present in the basic pattern below the lilac bands becomes intensified. The nature of this seasonal change is not yet understood, but it is probably due to metabolic changes connected either with preparations for hibernation or the sex-cycle.

An observation of interest that has arisen from this work is that those species which show a bright blue band at Rf 0.4-0.45 are all in varying degree susceptible to infection with *Fasciola gigantica* and refractory to *F. hepatica*. This species-group host-parasite relationship will almost undoubtedly be found to occur amongst the intermediate hosts of schistosomiasis, but here the problem is complicated by the necessity for using modified techniques for the detection of fluorescent patterns. It is certain that results similar to those given by the lymnaeid snails may be encountered in other groups; it is therefore to be hoped that no great taxonomic significance will be attached to isolated sets of chromatographic patterns which do not take into consideration either the possibly fine distinctions between separate species or, alternatively, the possible variations between populations of the same species or seasonal variations within a single population. Even if the technique has taxonomic limitations, it may still provide a general screening test to decide whether a population of snails is likely to prove suitable for the completion of a fluke life-cycle or not and this, from the epidemiological point of view, is obviously one of the most important characteristics of snails.

Once a miracidium has located and penetrated into a snail a new ecological phase of the parasite life-cycle begins. Even if the miracidium has selected an apparently suitable host, it may be that no further development will occur after penetration into the snail. This may be due to a natural resistance as described and investigated by Newton (1952) or it may be due to the physiological state of the snail. There is evidence from entomological work that there are species-specific differences in the free amino-acids of insect blood, also that there can be variations in the free amino-acid picture obtained from insects of the same species according to the physiological state of the individuals examined. Thus the pattern from hibernating mosquitoes shows differences from that given by recently emerged, unfed adults of the same species (Micks, 1954). It is possible that the investigation by means of two-dimensional paper chromatography of

the free amino-acids and peptides of snail tissues will yield not only further taxonomic characters of use in snail identification, but may also throw light on the ecology of the larval phases of trematodes while they are parasitic within their molluscan hosts. This type of study is complicated by the low concentrations of free amino-acids present in the blood of fresh-water animals compared to the amounts found in marine forms (Duchâteau *et al.*, 1952). The work of Pratt and Auclair (1948) has shown that sensitivity of amino-acids to the ninhydrin reaction on paper is very variable, some substances requiring to be present in much greater quantity than others in order that they may be detected. This means that very carefully controlled quantities of material must be used to ensure that any differences observed are not artificial.

This same aspect of the problem is also being approached through the application of electrophoretic separation of snail blood proteins. The information available on species-specific differences in blood proteins is limited and the value of paper electrophoresis of human serum in clinical diagnostic work indicates the great range of possible variation within a species. These facts throw doubt on the taxonomic importance of results obtained using this approach if those results are not treated in a sufficiently critical manner, but the possible light which may be shed on larval trematode development in the parasitic phase will more than compensate for this.

In conclusion it must be made absolutely clear that the techniques described here can only be of value in taxonomic work if they are used in conjunction with established methods based on anatomical and conchological investigations. In the same way that anatomical studies must be referred to observations on shell characters so must the results of biochemical analysis be considered together with morphological data. Attempts to introduce a classification of the mollusca based on the retention of a mucus smear and the discarding of the rest of the animal should be discouraged.

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NATURAL AND EXPERIMENTAL INFECTION OF SOME BULINID SNAILS IN THE SUDAN WITH *SCHISTOSOMA* *HAEMATOBIIUM*

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The writer (1956) recorded the distribution of snails of the genera *Bulinus* and *Biomphalaria* which are actually or potentially concerned in the transmission of bilharziasis in the Sudan. In order to obtain an elaborate picture of all phases of the problem in the country, it was then projected to study the natural infection of these snails and to find experimental and epidemiological evidence for the transmission of human and animal schistosomes by these snail intermediate hosts. The present paper is the first in a series and deals with *S. haematobium* in bulinid snails. The species of *Bulinus* reported are: *B. (B.) truncatus*, *B. (B.) truncatus rohlfsi*, *B. (B.) forskalii*, *B. (Physopsis) ugandae* and *B. (Ph.) globosus*.

B. (Ph.) ugandae (Mandahl-Barth) occurs in the Nile basin, namely in the Bahrel-Jebel (including the Sudd region), in the swamps of the eastern Bahr-el-Ghazl and in the White Nile. Its susceptibility to *S. haematobium* has been questionable, and, in Uganda, Cridland (1955) failed to infect it with *S. haematobium*. *B. forskalii* (Ehrenberg), which has been suspected as a transmitter of urinary bilharziasis in various African countries, is also tested in these experiments. As a control *B. (Bulinus) truncatus* (Audouin), the recognized intermediate host of urinary bilharziasis, is used. This species is here con-

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sidered to include *B. contortus*, *B. dybowskii*, *B. innesi*. *B. (Ph.) globosus* and *B. (B.) rohlfsi* were not available for experimental infection.

MATERIAL AND METHODS

Miracidia of *S. haematobium* were hatched from urine obtained from patients near Khartoum. Both river and well water were used in the hatching processes. The urine was placed in a 1 l flask provided with a side arm, as advocated by McMullen and Beaver (1945), and as used by the writer (1950) and some other workers. Water was then added to fill the flask and the side arm. It is born in mind that not all the miracidia collect in the side arm, but the number that collected there was enough for the purpose of the experiments.

The snails were placed individually or in groups of two or three in small specimen tubes, 12 mm in diameter, and the water with the miracidia placed in the tubes to a height of about 10 mm. From 6 to 8 miracidia per snail were pipetted into the tube. The exposure time was usually about 5 hours. Snails were examined for their shedding of cercariae starting the third week after exposure. Placing the bottles in the sun or in a warm shady place stimulated the emergence of cercariae.

Percentages of infections were calculated as to the number of snails which survived the incubation period, this period being that from the exposure to the miracidia to the first shedding of cercariae.

Both wild and laboratory-reared snails were used in these experiments. The wild snails were checked for natural infection with any trematode. Only those free from trematodes, judging only from their failure to shed cercariae when examined repeatedly, were used.

RESULTS

The results of examination of some species of bulinid snails in the Sudan for their natural infection with schistosomes are presented in Table 1. Seven hundred *B. (Physopsis) ugandae* from the White Nile shed only cercariae of trematodes other than schistosomes. 5.3 percent of *Bulinus truncatus*, also from the White Nile, shed schistosome cercariae. Three of the infected snails survived until white

mice were available for infection. Adult schistosomes recovered on autopsy of the mice, 95 days after their infection, proved that 2 of the snails were shedding *S. haematobium* cercariae and the third snail was infected with *S. bovis*. 1.7 percent of *Bulinus truncatus* from Shambat irrigation canals were shedding schistosome cercariae. When these cercariae infected mice, adult *S. haematobium* worms were recovered on autopsy. The percentage of infected *B. truncatus* in White Nile is based on examinations in the month of January. In May, however, the percentage of infected snails is higher (research in progress).

TABLE 1 — Results of examination of certain species of *Bulinus* in the Sudan for their natural infection with *Schistosoma haematobium*

Species	Locality	Height in millimetres	Number examined	Number shedding schistosome cercariae	Percentage of infected snails
<i>B. (Physopsis) ugandae</i>	White Nile	3-7	700	0	0
<i>B. truncatus</i>	White Nile	2-9	150	8	5.3
<i>B. truncatus</i>	Shambat	2-9	400	7	1.7
<i>B. forskalii</i>	White Nile	2-12	200	0	0
<i>B. forskalii</i>	Shambat	2-9	300	0	0

B. forskalii did not show any natural infection with schistosomes when 300 snails from Shambat agricultural scheme and 200 from the White Nile were examined.

Table 2 contains the data obtained in the experimental exposure of certain bulinid snails in the Sudan to the miracidia of *S. haematobium*. *B. truncatus*, both wild and laboratory-bred, was readily infected as follows: 62 wild snails from Shambat out of the 191 that survived, or 32.4 percent; 107 laboratory-bred (Shambat stock) out of the 270 surviving snails, or 39.6 percent; and 21 laboratory-bred (White Nile stock) out of the 98 surviving snails, or 21.4 percent. The daily output of cercariae from each infected snail is high and some snails have been shedding cercariae for over two months.

111 wild and 144 laboratory-bred specimens of *B. (Physopsis) ugandae* shed no cercariae and on examination of some of those which

died no sporocysts were found. However, when another batch of the same species was exposed to the miracidia, 6 snails out of the 117 survivors, or 5.1 percent, shed cercariae. Every snail shed a large number of cercariae every day for over two months.

Bulinus forskalii snails laboratory-bred of mixed stock were not infected when exposed to the miracidia. However, later in another experiment the same species showed the following infection rates: 6.9, 3.1 and 9.5 percent. It was noted that snails of this species that became infected showed a very low daily output of cercariae, lasting for only from 3 to 12 days.

The range of days from exposure to the miracidia to the first shedding of cercariae, i.e., incubation period, was 39-51 for *B. truncatus*, 42-54 for *B. forskalii*, and 62-65 for *B. (Ph.) ugandae*. The snail maintenance temperature was 24-27°C.

DISCUSSION

The first batch of *B. (Ph.) ugandae* (Table 2) were not infected on exposure to the miracidia of *S. haematobium*. (Unfortunately about 60 percent of this batch did not survive the incubation period.) However, 6 snails out of the 117 survivors of the second batch became infected and shed a large number of *S. haematobium* cercariae for long periods. There is therefore experimental evidence that in nature this bulinid snail is most probably a vector of *S. haematobium*, though susceptibility may be low. Moreover, the White Nile area offers epidemiological evidence which supports the positive results of the present exposure experiment.

When more *Bulinus (Physopsis) ugandae* snails were later collected at low water from the White Nile (during the month of May), 2 snails were shedding schistosome cercariae out of 73 collected. One of these snails had a mixed infection with an amphistome. When mice were exposed to these cercariae and autopsied 90 days later, adult *Schistosoma bovis* worms were recovered. The identification was made on the basis of the in utero eggs.

Cridland (1955) could not infect *B. (Ph.) ugandae* from East Africa. In the Sudan, *B. (Ph.) africanus*, *B. (Ph.) globosus*, *B. (Ph.) didieri* and *B. (Ph.) eximia* were reported by Archibald and Marshall

(1932) and by Archibald (1933) not to be susceptible to infection with *S. haematobium* in their experiments. *B. (Ph.) didieri* is synonymous with *B. (Ph.) globosus*, according to Mandahl-Barth (1958).

TABLE 2 — Results of exposure of certain species of *Bulinus* in the Sudan to miracidia of *Schistosoma haematobium*

Species	Locality	Age of snail in weeks	Number of snails exposed	Snails surviving incubation period		Surviving snails shedding cercariae		Incubation period in days
				Number	Percent	Number	Percent	
<i>B. truncatus</i> . . .	Shambat wild	2-4 *	220	191	86.8	62	32.4	43-50
<i>B. truncatus</i> . . .	Shambat	2-4	385	270	70.1	107	39.6	39-46
	laboratory-bred							
<i>B. truncatus</i> . . .	White Nile	2-4	170	98	57.6	21	21.4	42-51
	laboratory-bred							
<i>B. (Physopsis) ugandae</i> . . .	White Nile wild	3-6 *	210	111	52.8	0	0	—
<i>B. (Physopsis) ugandae</i> . . .	Laboratory-bred	2-5	345	144	41.7	0	0	—
<i>B. (Physopsis) ugandae</i> . . .	Laboratory-bred	2-3	180	117	65	6	5.1	62-65
<i>B. forskalii</i> . . .	White Nile	1-4	190	86	45.2	6	6.9	45-49
	laboratory-bred							
<i>B. forskalii</i> . . .	Shambat wild	2-4 *	150	95	63.6	3	3.1	42-48
<i>B. forskalii</i> . . .	Shambat	1-4	270	147	54.4	14	9.5	45-54
	laboratory-bred							
<i>B. forskalii</i> . . .	Mixed stock	1-4	460	314	68.2	0	0	—
	laboratory-bred							

* Approximate age.

Cram *et al.* (1947) were able to infect *B. (Ph.) africanus* in the United States, and Gordon *et al.* (1934) in Sierra Leone infected *B. (Ph.) globosus*. Vogel (1932) was unable to infect *B. (Ph.) globosus* in Liberia and French Guinea.

As for *B. truncatus*, specimens naturally infected with *S. haematobium* were encountered in the White Nile and in irrigation canals at Shambat (Table 1). Moreover, high infection rates (32.4, 39.6 and 21.4 percent, Table 2) were obtained experimentally. It is of interest to note here that, although it has been reported by some investigators that it was difficult for them to infect *B. truncatus* with *S. haematobium*, Moore *et al.* (1953) have overcome this difficulty by infecting 1-7 day-old snails, the most susceptible. In the present work this was made use of, and a large number of snails were infected and made available for experimental work.

Table 2 shows that *B. forskalii* can be experimentally infected with *S. haematobium*, though no infection was obtained at the beginning of the experimental work and no natural infection with schistosomes was encountered in the area investigated (Table 1). The experimental evidence of the present investigations would incriminate *B. forskalii* as a transmitter of *S. haematobium* in the Sudan, although conclusive evidence is still lacking. But even if a number of snails of this species will be found naturally infected, they would not be an important factor in the spread of the disease: those snails which became infected experimentally shed very few cercariae, and for only a short period. There is also some indication that infection of this species would not occur in nature in several habitats. Wright (1956) reported on the habitat preference of this species, being found at the bottom and thus does not promote the negatively geotropic miracidia to come in contact with it. This species has been suspected of transmitting urinary bilharziasis in several countries. Adams (1934, 1935), in Mauritius, found experimentally that *B. forskalii* could transmit *S. haematobium*; this called attention to this species in various African countries. The subject is summarized by Cowper (1953) and by Kuntz (1955). Naturally infected snails encountered in those countries were shedding mammalian-type schistosome cercariae only; there was no definite identification of *S. haematobium* in any naturally infected *B. forskalii*. McClelland (1955) found *B. forskalii* naturally infected with a bovine schistosome in western Kenya, and Le Roux (1955b) refers to cercariae from the same snail in the same area just as «mammalian schistosome cercariae». Le Roux (1955a) recovered a *Schistosoma* sp., probably *S. bovis*, from a goat at Kisumu and

believes that *B. forskalii* may also prove a vector of the blood fluke of man in that area.

One of the common snails in the White Nile area is *Lymnae natalensis*, but the possibility of its incrimination in the transmission is excluded. Negative results were obtained when 150 small and medium-sized snails were exposed to the miracidia of *S. haematobium*. Moreover, in a routine examination of these snails for their infection

TABLE 3 — *Distribution of bulinid snails and percentage of S. haematobium in various provinces of the Sudan*

Province	Bulinid snail				Percentage of <i>S. haematobium</i>	
	<i>Truncatus</i>	<i>Forskalii</i>	<i>Ugandae</i>	<i>Globosus</i>	1949	1952-1953
Northern	**	*				5.8
Wadi Halfa					8.1	
Merowe/Dongola . .					6.5	
Khartoum	**	*			1.7	1.7
Blue Nile						
Gezira	**	*			8.9	
White Nile.	*	*	**		5.8	5.4
Kordofan	**	*			22.8	14.1
Darfur	**	*			17.8	12.2
Upper Nile	*	*	**		2.5	2.1
Bahr-el-Ghazal . .	×	*	**		0.25	3 imported cases
Equatoria		*	*	*	none	

* Present.

** Predominant.

× Rare.

with *Fasciola gigantica*, they shed only cercariae of trematodes other than schistosomes.

In the Sudan *S. haematobium* is not prevalent or is absent in the southern provinces; it is not as common in the White Nile as it is in the Gezira or in the western provinces. It is intended in this discussion to find an explanation for such a distributional pattern, on the bases of one or more of the various factors which influence the distribution of schistosomiasis in general (snail factor, human factor, geographical factor). Table 3 represents a correlation of the distri-

bution of bulinid snails in the Sudan and their susceptibility as indicated in the present experiments, with the percentages of *S. haematobium* as indicated in the results of examinations of urine in government hospitals. These results appear in the Reports of the Sudan Medical Services, for example those of 1949 and 1952-1953. In the Table the predominance of *B. (Ph.) ugandae* is associated with a low incidence of *S. haematobium*. In the White Nile area the transmission of *S. haematobium* is apparently carried out by *B. truncatus* and probably to some extent by *B. (Ph.) ugandae*. The low figure for *S. haematobium* in Upper Nile province may be explained by the observation that the density of *B. truncatus* is gradually reduced as one proceeds south in the White Nile and Bahr-el-Jebel.

The geographical factor is also apparent and provides a sound explanation. Upper Nile province is mostly in the form of extensive swamps which are not densely inhabited and are a barrier to population movements. Upper Nile lies outside the main transit routes of immigrants, laborers and pilgrims from the north and west, the swamps forming a buffer zone, between a northern mixed, but predominantly vesical, bilharziasis zone, and a southern zone where intestinal bilharziasis prevails. This view was also presented by Archibald (1933) and confirmed by Ayad (1956). The dense tropical forests in Southwest Sudan is probably also a limiting factor for urinary bilharziasis. Intestinal bilharziasis is, however, prevalent there.

Two other aspects should not be overlooked: evidence has been accumulating on the competition with *S. haematobium* by *S. bovis* and other trematodes in *B. (Ph.) ugandae*, and that in the Sudan there is probably more than one strain of *S. haematobium*, to some of which *B. (Ph.) ugandae* may not be susceptible.

SUMMARY

1. Certain bulinid snails in the Sudan were examined for their natural infection with schistosomes; their susceptibility to infection with *S. haematobium* was tested by their exposure to the miracidia.

2. *Bulinus truncatus* appears to be the main intermediate host of urinary bilharziasis in the Sudan. High infection rates were obtained experimentally, and naturally infected snails were encountered shedding *S. haematobium* cercariae.

3. Although no naturally infected *B. (Physopsis) ugandae* were collected, experimental and epidemiological evidence suggests this species as an actual transmitter of *S. haematobium* though susceptibility is lower than *B. truncatus*. Two snails were later found naturally infected with *Schistosoma bovis*.

4. *B. forskalii* is probably not an important transmitter, due to the fact that the snails which became infected shed only a few cercariae for only a short period, after which infection died out.

5. A correlation was attempted of the distribution of bulinid snails in the Sudan and the results of the present work with the prevalence of *S. haematobium*, as indicated in government reports. Geographical and human factors affecting transmission are discussed.

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STUDIES ON THE BIOLOGY AND CONTROL OF SCHISTOSOME-BEARING SNAILS¹

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After the second World War, schistosomiasis became recognized as one of the most important health problems in many tropical and subtropical regions. Despite the fact that mass control measures against schistosomiasis were started forty years ago, none has been notably successful in abating transmission. Concerted effort in schistosomiasis control was quite naturally directed towards the development of molluscicides. The failure of chemicals to eradicate snails emphasized the need for comprehensive information on the biology and ecology of the schistosome-bearing mollusca. The possibility of control by biological means has, at times, been mentioned, but this area never received more than cursory attention. The experiments summarized here were initiated in 1953 and were designed to gain information on this subject.

An initial review of more than 600 publications dealing with parasites and predators of snails failed to provide a rational basis for selecting specific organisms for study as control agents (1). We focused our attention on possible bacterial pathogens and predators. A knowledge of normal snail histology was of paramount

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importance for the recognition of pathological processes which might be induced by microorganisms. Therefore, a detailed study was undertaken of the general histology and topographic microanatomy of *Australorbis glabratus*, the vector of *Schistosoma mansoni* in the Western Hemisphere (2).

Two microorganisms were encountered in the tissues of supposedly «normal» individuals during histological studies on *Australorbis*. One is a fungus-like organism which invades various tissues, but occurs particularly in the nerve ganglia. It appears to provoke proliferation of the phagocytic amoebocytes which are commonly found in lesions walled-off by layers of fibroblasts. Mycelial growth has also been observed in the loose vascular connective tissue.

The second is an acid-fast bacillus which appears to be an obligate intracellular organism. It has always been found in dense masses within aggregated amoebocytes which are surrounded by a well-marked fibrous capsule. The parasitized amoebocytes occur in a wide variety of tissues—the rectal ridge, kidney, gut, mantle, foot and ovotestis. Cultural isolates, believed to represent this organism, have been successfully made and are under study (3).

More recently, discrete, yellowish, macroscopically-evident, disseminated lesions were discovered in our colony of a local snail, *Helisoma anceps*. Microscopically, the lesions consist of aggregates of infected amoebocytes, resembling those observed in *A. glabratus*. There is evidence that mortality among infected snails is high and that reproduction is impaired. This organism has also been isolated in pure culture and transfer to presumably normal snails has been accomplished.

Early in our studies it was realized that certain problems could be approached only if snails could be secured free of microbial contaminants. Experiments were undertaken in an effort to surface-sterilize mature egg-masses of *A. glabratus* and to have them hatch in a sterile saline solution. Hatching did occur, but a variable and unpredictable proportion of the individual egg-masses were contaminated. It was ultimately discovered that otherwise normal eggs occasionally contain protozoa, and presumably other contaminants as well, which could not be destroyed by surface treatment and which were released from the egg upon hatching. A method was then devised to separate large numbers of eggs from each other by

manual dissection under a stereomicroscope. Groups of such eggs are subjected for 10 minutes to a dilute solution of sodium hypochlorite, washed, and transferred to individual tubes containing sterile saline and antibiotics, sealed and maintained at 28°C. The hatch rate is of the order of 90-100 percent within 3-4 days and from 90-100 percent are bacteriologically sterile. The newly-hatched snails grow readily under axenic conditions when fed a mixture of autoclaved brewer's yeast and formalin-killed *Escherichia coli*. A maximum size of 6 mm has been obtained (4, 5, 10).

An understanding of population dynamics of schistosome-bearing snails was felt to be fundamental to eventual control. Accordingly, studies were undertaken to determine the effects of crowding on populations of *A. glabratus*. Let it suffice to say that crowding resulted in a reduced rate of growth of the individuals, as judged by mean shell diameter, and that it also had an adverse effect on the snail's fecundity. Our observations here appear to have added significance in view of the fact that species and subspecies of snails have at times been identified or erected using shell size as a criterion. Further, the increased growth and fecundity of small populations of snails may serve to explain the rapid re-population of areas subjected to control measures in the field (6, 7).

Our attention was drawn to field observations by Dr. José Oliver-González suggesting that a recently imported ampulariid snail, *Marisa cornuarietis*, might be responsible for the disappearance of *A. glabratus* from a stream in Puerto Rico. It was suggested that our group investigate the mechanism which might be involved. Our experiments showed conclusively that *Marisa*, a large snail, is capable of controlling both *A. glabratus* and *Biomphalaria pfeifferi* under laboratory conditions. *Marisa* is a voracious herbivore and in the course of feeding on aquatic vegetation ingests the egg-masses and newly-hatched snails of these two schistosome vectors. The practical use of *Marisa* to control schistosome-bearing snails in nature requires much further study in view of the possibility of damage to crops grown in water. This particular snail in a paddy field might cause grave calamity (8, 9).

During the studies on axenic cultivation of snails it was discovered that streptomycin in the medium either retarded or completely inhibited growth and reproduction of *A. glabratus*. This observation

led to studies of the effects of the antibiotic on snails under aquarium conditions. It was observed that, upon exposure to a small initial dose of streptomycin, snails immediately drop to the bottom of the aquarium, twist and gyrate, and appear incapable of directed movement for about 2-4 days. Despite the presence of ample food, growth ceases. Reproduction was almost completely inhibited when breeding adults were maintained for several weeks in aquaria containing streptomycin (10). The presence of streptomycin also inhibits the hatching of *A. glabratus* eggs. However, the behavioral pattern of the adult snails and the inhibitory effect on hatching of eggs were prevented by the addition of inorganic ions, particularly calcium ions (CaCl_2), to the aquarium and the culture medium, but had little or no effect on the concurrent growth-inhibition induced by the antibiotic.

In our study we have had many questions, but we have very few answers. It is a «beginning» — and it must be admitted that there is much to be learned about the biology of snails before practical biological means of control can be applied. In his Charles Craig Lecture, Dr. Henry Meleney aptly remarked: «We are now in a stage in the conquest of schistosomiasis comparable to the stage in malaria control when we had only quinine for treatment, oil, Paris green and ditching for larva control, and fly swatters and screening for adult mosquitoes.» (11). This was said four years ago — in the interim, studies on the biology and ecology of fresh-water snails have been pursued eagerly in many and widely separated laboratories. Time will be the important factor in producing answers for the questions.

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CHARACTERISTICS OF GEOGRAPHIC STRAINS OF *SCHISTOSOMA JAPONICUM* IN THE FINAL HOSTS¹

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Schistosoma japonicum was first described in 1904 by Katsurada in Japan. In the same year, Catto found this fluke in an autopsy case of a Chinese who came from Fukien, China, but died at Singapore. Catto's fluke was named by Blanchard as *Schistosoma cattoi* (Catto, 1905). After comparing the descriptions of the Japanese and Chinese forms, Stiles (1905) declared that they are identical. Subsequently, *S. japonicum* was reported by Logan (1905) in China, Wooley (1906) in the Philippines, Takegami (1914) in Formosa, and Brug and Tesch (1937) in Celebes. No differences in the parasite from different geographic areas have been noticed.

In reviewing the literature concerning schistosomiasis japonica in Formosa, we (Hsü *et al.*, 1952) were first to point out that although *S. japonicum* has been reported in domestic animals in Formosa, no human case has hitherto been verified. The results of our subsequent researches (Hsü, Hsü, and Ritchie, 1955; Hsü and Hsü, 1956) have indicated the possibility that *S. japonicum* in Formosa is a non-human strain.

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Recent studies made in our laboratory indicate that *S. japonicum* has four well defined geographic strains, the Chinese, Japanese, Formosan, and Philippine. The present paper is a summary report on their characteristics.

RESULTS

(1) *Host specificity.* We have demonstrated that stool examinations of 4,197 persons by the sedimentation method in the main endemic area of schistosomiasis in Formosa did not reveal a definite positive case of *S. japonicum* infection (Hsü, Hsü, and Ritchie, 1955). We have also shown that stool examinations and liver biopsies were negative for *S. japonicum* eggs in 5 human volunteers who had been exposed to 400 to 800 cercariae of the Formosan strain (Hsü and Hsü, 1956). It has been concluded that the Formosan strain of *S. japonicum* is a non-human zoophilic strain.

Results of experimental infections with schistosome cercariae to 36 monkeys showed that the Formosan monkey, *Macacus cyclopis*, was highly susceptible to the Japanese and Philippine strains, but was a relatively poor host for the Formosan strain (Hsü and Hsü, 1956a). Although the rhesus monkey, *Macacus rhesus*, is known to be highly susceptible to the Chinese strain (Vogel and Minning, 1953), recent studies in our laboratory show that this monkey species is refractory to the infection of the Formosan strain (Hsü and Hsü, unpublished data). This indicates that the Formosan strain of *S. japonicum* differs from the Chinese, Japanese, and Philippine strains in infectivity in certain species of the Primates.

(2) *Size of the worm.* The mature worms obtained from rabbits and hamsters infected with the Formosan and Japanese strains showed that both the male and female specimens of the Formosan strain were significantly longer than those of the Japanese strain (Hsü and Hsü, unpublished data).

(3) *Number and location of testes in the male worm.* The number of testes in the male *S. japonicum* has been found to vary from 1 to 14. The percentages of the specimens with 7 testes in the different strains are: Japanese, 95.4; Chinese, 91.9; Formosan, 88.4; and Philippine, 77.4. The Chi-square test shows that the percentages of specimens with 7 testes are significantly different between all the

strains, except in the case of the Chinese and Formosan (Hsü and Hsü, 1957).

In certain specimens one or several testes may be situated in an unusual location. The percentage of testes in unusual locations is 9.8 for the male specimens obtained from rabbits infected with the Formosan strain, whereas it is only 1.5 for the males obtained from rabbits infected with the Japanese strain. The difference has been shown to be statistically significant (Hsü and Hsü, 1957).

(4) *Hermaphroditic males*. In the examination of 1,434 male specimens of the Formosan strain, 345 specimens of the Chinese strain, 684 specimens of the Japanese strain, and 84 specimens of the Philippine strain, 6 hermaphroditic ones were found. All were obtained from animals infected with the Chinese strain (Hsü and Hsü, 1957).

(5) *Size and shape of the eggs*. The measurements of the eggs of *S. japonicum* from dogs, cats, hamsters and albino mice infected with the Chinese, Formosan, Japanese, and Philippine strains have been compared. Between strains, if the mean of the measurements of a number of eggs from a host-individual was obtained and the collective means from a number of host-individuals infected with one strain were compared with those of the other strains, significant inter-strain differences were found in the length, width and especially in the index of the eggs. The confidence intervals of the mean of the means of the index of the eggs were very distinct, i.e., there was no overlapping among the four strains. The indices of eggs of the four strains fell into the following order: Japanese > Formosan > Philippine > Chinese. The eggs of the Japanese strain were broad and short, giving a high index. Those of the Chinese strain were narrow and long, giving the lowest index. The other two strains lay between these two extremes, in the order given above (Hsü and Hsü, 1958).

(6) *Prepatent period*. The prepatent period of the four strains of *S. japonicum* has been determined in cats, dogs, hamsters, Formosan monkeys, mice, and rats. Among the host-species which are susceptible to all the strains, i.e., the cat, dog, hamster, rabbit, and mouse, the means of the prepatent period in different host-species were from 37 to 44 days for the Formosan strain; from 38 to 47 days for the Chinese; from 34 to 48 days for the Japanese; and 35 days for the Philippine strain. In general, this period was longer in the

Chinese and Formosan strains than that in the Japanese and Philippine strains.

Special experiments have been made for a more detailed study on the prepatent period of the four strains in albino mice. The mean and standard error of the prepatent period of the Chinese strain was found to be 42.1 ± 0.53 days, 41.4 ± 0.38 for the Formosan, 34.7 ± 0.23 for the Japanese, and 35.5 ± 0.43 for the Philippine. It will be noted that the prepatent period of the four strains also fall into two categories: (1) the short period group (mean, 35 to 36 days), to which the Japanese and Philippine strains belong; and (2) the long period group (mean, 41 to 42 days), to which the Chinese and Formosan strains belong (Hsü and Hsü, 1958a).

(7) *Distribution of eggs in various organs of the host.* The distribution of the schistosome eggs in various organs of albino mice infected with four geographic strains has been studied. The preliminary results show that for the Chinese strain the relative percentages of the egg distribution in various organs are: lungs 0.1, liver 42.8, spleen 0.2, stomach 0.2, small intestine 33.8, and large intestine 22.9; for the Formosan strain: lungs 0.05, liver 43.6, spleen 1.6, stomach 1.0, small intestine 40.0, and large intestine 13.7; for the Japanese strain: lungs 0.001, liver 11.1, spleen 0.1, stomach 0.1, small intestine 81.1, and large intestine 7.6; and for the Philippine strain: lungs 0.05, liver 23.9, spleen 0.01, stomach 0.4, small intestine 59.7, and large intestine 6.0. When the egg distributions in the organs from the hosts infected with different strains are compared, the Chinese strain is characterized by the highest percentages of the eggs in the lungs and large intestine; the Formosan strain, by the highest percentages of the eggs in the liver, spleen, and stomach; the Japanese strain, by the highest percentages of the eggs in the small intestine; and the Philippine strain, by the moderately high percentages of the eggs in the small and large intestine (Hsü and Hsü, unpublished data).

(8) *Virulence.* The virulence of the Chinese, Formosan and Japanese strains of *S. japonicum* has been studied in the albino mouse. 36 mice have been infected with each strain. Each mouse was infected with 50 male and 50 female cercariae. After the appearance of eggs in the stools, the mean life span of the infected animals was 7.9 days for those infected with the Formosan strain, 6.9 days with the Chinese strain, and 4.5 days with the Japanese strain. The difference

in means of the life span is not significant between the Chinese and Formosan strains, but very significant between the Chinese and Japanese strains, and between the Formosan and Japanese strains. The results indicate that in mice the Japanese strain of *S. japonicum* is more virulent than the Chinese and Formosan strains (Hsü and Hsü, unpublished data).

DISCUSSION

It has been well established by the above study that *S. japonicum* from each of the four major endemic areas, China, Formosa, Japan, and the Philippines, possesses distinct characteristics and that at least four geographic strains exist. It is of interest to note that *S. japonicum* from Kyushu and Yamanashi, two well separated endemic areas in Japan, shows no significant differences in the pre-patent period and in the size and shape of the eggs in albino mice (Hsü and Hsü, 1957a).

As the four geographic strains are distinguished mainly biologically, it is hardly possible to consider them as four morphological species. Two working hypotheses are possible to account for the differences among the four geographic strains. Either they are four sibling species which differ in their biological properties, but only slightly in their morphology, or else they are four geographic races or subspecies of a single polytypic species. Experimental interbreeding of different geographic strains will reveal the amount of their genetic divergence. If the fertility among the four strains is only reduced, they should be regarded as four subspecies. If the four strains are completely sterile or virtually sterile, they are entitled to be regarded as sibling species.

Although each of the four geographic strains of *S. japonicum* shows its distinct characteristics, we believe that they were descended from a common ancestor. In his classic book of zoogeography, Darlington (1957) asserts that the main pattern of dispersal of animals is apparently due to the evolution of successive dominant groups in the great, favorable area of the main part of the Old World tropics and spread into smaller and/or less favorable areas, with successive replacement. He says further that the animals of continents are apparently always dominant over those of islands, and dispersal is almost always

from continents to islands. It is reasonable to suspect that *S. japonicum* has followed this general pattern. We believe that *S. japonicum* originated in the vicinities of South China and from there radiated into Central China, Japan, Formosa, Celebes, and the Philippines. The dispersion should have been completed before the islands were separated from the Asiatic continent, i.e., before or in the Pleistocene. During the process of evolution, this parasite was constantly multiplying in some places, spreading into other places, receding and becoming extinct in others, and thus forming the present apparently discontinuous pattern of geographic distribution. Mutations will occur in the isolated populations. As the survival and spread of mutations may proceed most rapidly in relatively small, isolated populations, this kind of evolution will produce new strains in these islands. The mutations that happen to be advantageous under special conditions are selected. From the Pleistocene to the present period, it has been about a million years. *S. japonicum* on these islands should have undergone sufficient reproduction isolation, ecological differences, and general adaptation, to have characteristics distinct from its ancestral form in the Chinese mainland. As the ecological conditions and general adaptations were different in different isolated areas, the characteristics of various strains in different islands, i.e., Japan, Formosa, Celebes, and the Philippines, should have been developed differently. Physiological differentiations of isolated populations sometimes proceed more rapidly than morphological changes (Mayr, 1957). The insular strains of *S. japonicum* have, indeed, become very distinct in physiological characteristics. However, we have also made a beginning of finding certain minor morphological differences in them, and it is probable that search of features not yet adequately explored will reveal more, especially in cytologic differences at the level of electron microscopy.

The survival and subsequent proliferation of a parasite in a new area during dispersal is not a simple matter. In the case of *S. japonicum*, in addition to the necessity of the presence of a proper final host, an intermediate host, the oncomelanian snail, is required. Although the final hosts of this parasite, man, domestic mammals, and rodents, are usually present in all the endemic areas, species of the oncomelanian snails have a special geographic distribution. Differences in the infectivity of the various geographic strains of

S. japonicum have been demonstrated with the oncomelanian species from different endemic areas (Hunter *et al.*, 1952; DeWitt, 1954). The effect of local oncomelanian species on the development of the characteristics of the local strain of the parasite should be considered.

The attempt to determine the characteristics of various geographic strains of *S. japonicum* has led in many cases not only to a more precise formulation of a problem, but very often also to its solution. The results of our studies on the characteristics of the Formosan strain have led to the discovery of a non-human strain of this parasite. In their studies on schistosomiasis japonica in China, Faust and Meleney (1924) reported that in their experimental dogs the jejunum was the portion of the alimentary tract most intensely involved, and the lesions were progressively fewer in number through the ileum towards the colon. They were puzzled by another finding that in human cases the distribution of the intestinal lesions was distinctly different from that in their dogs because the colon was the part most involved. At that time, the importance of geographic strains in *S. japonicum* was not known. In their interpretive discussion, they did not consider the fact that their dogs were infected with the cercariae from *Oncomelania nosophora* from Japan and their autopsied Chinese patients were infected with the cercariae of the Chinese strain. The results of our studies on the distributions of eggs in various organs of the infected show that in the mice infected with the Japanese strain, 81 percent of the eggs were found in the small intestine, especially in the jejunum, and only 8 percent in the large intestine; whereas in the animals infected with the Chinese strain, only 34 percent of the eggs in the small intestine, but 30 percent in the large intestine. As the intensity of the lesions in various organs in the hosts infected with *S. japonicum* usually bears a direct relationship to the number of eggs deposited in the respective organs, the pattern of the distribution of the lesions in the digestive tract will certainly differ in the hosts infected with the Japanese and Chinese strains. The patterns of the distributions of lesions in the digestive tract of the rhesus monkey infected with the Chinese strain (Vogel and Minning, 1953) and of the mouse and rabbit infected with the Japanese strain (Ito, 1955) agree well with the pattern of the lesions produced by the respective strain in the mouse as described above. This problem will be fully treated again in our subsequent publication on this subject.

We would like to emphasize that since each geographic strain of *S. japonicum* possesses distinct characteristics, further studies on the pathogenesis produced by this parasite have to be made on the strain basis.

SUMMARY

Four geographic strains of *S. japonicum*, Chinese, Formosan, Japanese, and Philippine, were used for making comparative studies of the following strain characteristics: (1) host specificity, (2) size of the worm, (3) number and location of testes in the male worm, (4) hermaphroditic males, (5) size and shape of the eggs, (6) prepatent period, (7) distribution of eggs in various organs of the host, and (8) virulence.

The existence of four geographic strains with distinct characteristics has been well established. All the four strains are assumed to have a common ancestor in South China. From there the parasite radiated into Central China, Japan, Formosa, Celebes, and the Philippines before or in the Pleistocene. As a result of the eventual isolation of the islands by effective barriers, each insular strain developed distinct characteristics of its own.

The attempt to determine the characteristics of various geographic strains of *S. japonicum* has led in many cases not only to a more precise formulation of a problem, but very often also to its solution. It was emphasized that since each geographic strain of *S. japonicum* possesses distinct characteristics, further studies on the pathogenesis produced by this parasite have to be made on the strain basis.

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RELATION OF SKIN REACTION TO PENETRATION
AND TO THE DEVELOPMENT OF LOCAL RESISTANCE
TO ENTRY BY CHALLENGING CERCARIAE OF *SCHIS-*
*TOSOMA MANSONI*¹

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Invasion of the epidermal surface of a receptive organism is the first step in the relationship of many parasites to their definitive hosts. This first contact is worthy of much more thoughtful consideration than it has heretofore received. It is a vulnerable spot in many parasitic life cycles. It is of especial interest both as a complex organism-environment interface which not only constitutes a physical barrier to parasites, but also presents a chemical problem to a would-be invader.

Examination of this tissue from the standpoint of the schistosome cercaria will serve to orient our thinking in a common direction. The epidermal surface is the third environmental condition to which this larval stage of the schistosome has had to adjust in a short period of time. The first was the tissue of the poikilothermic mollusc in which it developed and through which it recently migrated. The second was the water of the pond or stream into which it emerged.

This epidermal surface (Fig. 1) is covered with sebum. It presents a very deeply furrowed but fairly continuous wax-covered

¹ The opinions contained in this paper are those of the author and are not to be construed as necessarily reflecting the views or endorsement of the Navy Department.

surface which is interrupted by the points of exsertion of hair shafts, and in some areas also by sweat gland openings. The continuity may be further disrupted by scales, and by bits of collected material adherent to the surface (Stirewalt, 1956).



Fig. 1 — Abdominal skin of adult hamster. (From Stirewalt, 1956)

Chemically, the waxy film is homogeneous, fatty and aqueous phases forming a fine emulsion containing cholesterol, cholesterol esters and waxes. Through this surface heat must be radiated and chemical substances dispersed which may or may not be stimulatory

to cercariae. The influence of such factors on penetration by parasites has not been adequately studied. That some percutaneous absorption occurs through this waxy film has been shown for many substances including lipids and water. The swelling and softening of the horny layers beneath the film upon contact with water are well known.

Skin, however, is by no means the same, either on different areas of the same animal, at various ages, or on different animals. Variations in the surface presented were found to be reflected in the reactions of cercariae placed thereon (Stirewalt, 1953). For instance, relatively few cercariae penetrated skin of newborn mice as compared with that of older mice. Times spent in exploration over the surface and in entry into it varied with the skin of different animals. Average exploratory times were from less than 30 seconds on adult human skin to more than 4 1/2 minutes on 5-day-old rat skin. Average variations in actual entry time were from about 3 minutes on young mouse skin to 7 minutes on adult hamster skin. Nor were points of entry constant (Stirewalt, 1956). One hundred percent of the cercariae observed to enter human skin attacked in skin furrows, while 90 percent of the penetrants into hairless mouse tail skin entered in the region of hair follicles. Patterns of behavior differed as well. On the body skin of hairless mice, cercariae looped along briefly, alternately attaching by the two suckers and then orienting themselves for penetration, while on young rat skin they usually became tethered by mucoid secretions from the acetabular glands. These stretched into ropes with the movements of the cercariae. Such ropy secretions were not observed on other skin surfaces. It is possible that this may be correlated with the peculiar sebum of young rat skin.

Under the sebum film lie the keratinized cells of the corneal layer. Movement through this layer was the most time-consuming phase of entry. Successful passage through it appeared to result from a combination of mechanical effort and lytic action of secretion from the acetabular glands (Stirewalt, 1956). Such invasion of skin produced certain changes, some of which were observed macroscopically, others only microscopically. These changes and their relation to schistosome invasion have received special attention in our laboratory. By exposing mouse tail skin to known numbers of cercariae under conditions permitting no loss of parasites by splashing, fecal

TABLE 1 — Average maximum cercarial penetration per mouse from the challenging exposure presented at the indicated intervals after the initial exposure and compared with that of the controls. (From Stirewalt, 1953)

Interval between exposures	Description of schistosomes of initial infection	Cercarial penetration of challenging infection			
		Experimental		Control	
		No Mice	Av. No. Cerc. SE	No. Mice	Av. No. Cerc. SE
One hour	Larvae in skin	40	36.1* $\pm$ 1.1	20	45.2 $\pm$ 0.6
Five hours	Larvae in skin	30	29.9* $\pm$ 1.7	15	37.8 $\pm$ 1.4
One day	Larvae in skin	99	36.1* $\pm$ 0.8	50	43.4 $\pm$ 0.7
Two days	Larvae in skin	30	32.6 $\pm$ 1.3	15	36.9 $\pm$ 1.4
One week	Larvae migrating from skin	68	43.2 $\pm$ 0.8	35	42.3 $\pm$ 1.2
One month	Young worms in portal veins	130	44.9 $\pm$ 0.4	47	41.7 $\pm$ 0.7
Two months	Adult worms and eggs	46	40.9 $\pm$ 0.9	24	41.6 $\pm$ 1.1

* Statistically significant variation from control (p less than 0.001).

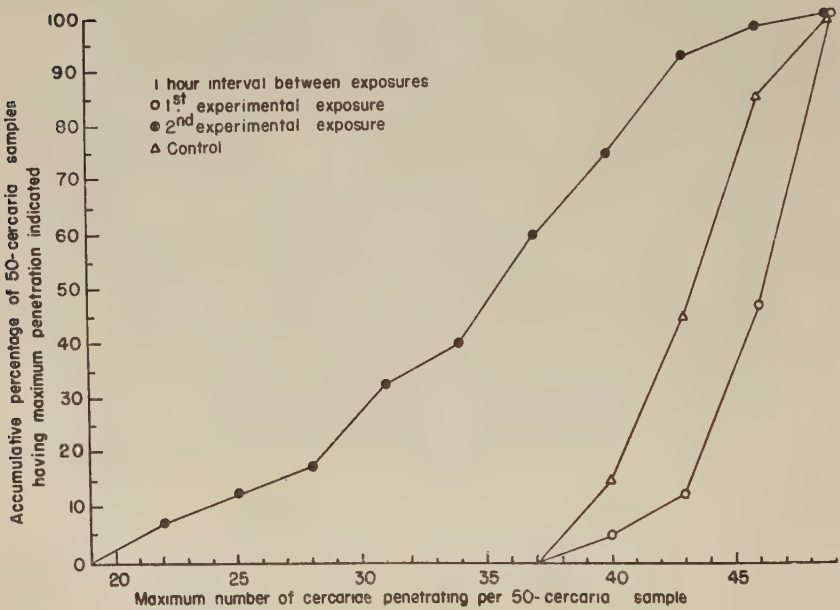


Fig. 2 — Accumulative frequency distribution showing percentage of 50-cercaria samples in which the maximum number penetrating was $\leq$ that indicated. Data are by class intervals of 3. (From Stirewalt, 1953)

TABLE 2—Average worm burden per mouse from the challenging exposure presented at the indicated intervals after the initial exposure and compared with that of control mice. (From Stirewalt, 1953)

Interval between exposures	Description of schistosomes of initial infection	Worm burden from challenging infection			
		Experimental		Control	
		No. Mice	Av. No. Worms SE	No. Mice	Av. No. Worms SE
One hour	Larvae in skin	30	6.6* $\pm$ 1.1	30	20.3 $\pm$ 1.4
One day	Larvae in skin	28	9.2* $\pm$ 1.1	30	17.9 $\pm$ 1.5
One week	Larvae migrating	30	19.1 $\pm$ 1.3	30	18.0 $\pm$ 0.8
One month	Young worms in portal veins	30	15.4 $\pm$ 0.9	30	13.4 $\pm$ 1.5
Two months	Adult worms and eggs	26	10.7* $\pm$ 1.1	27	17.1 $\pm$ 1.1

* Statistically significant variation from control (p less than 0.001).

contamination or dispersion over a large area of furred skin, and counting the cercariae not penetrating, it was shown that challenging

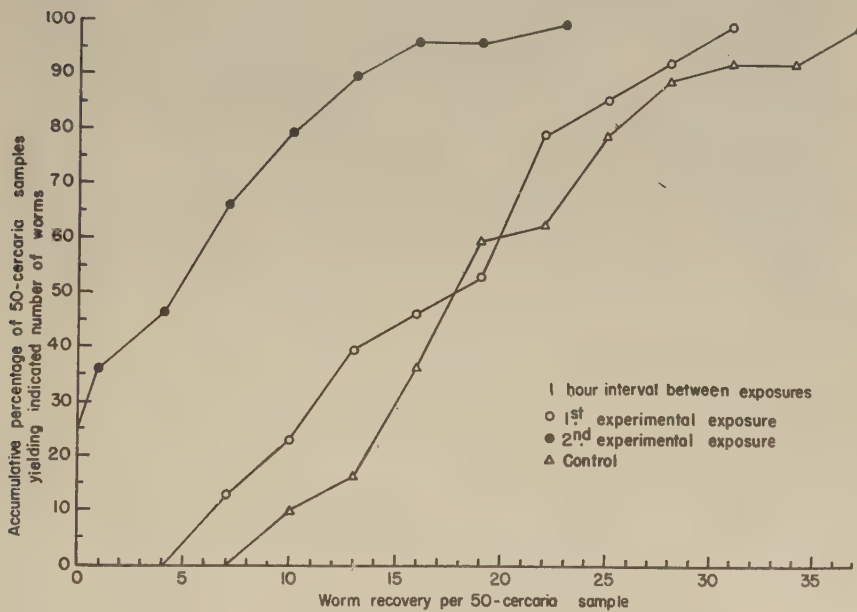


Fig. 3 — Accumulative frequency distribution showing percentage of 50-cercaria samples yielding $\leq$ the indicated number of adult worms in class intervals of 3. (From Stirewalt, 1953)

cercariae applied to this skin within 24 hours of an initial exposure, penetrated poorly, as may be confirmed in Table 1 (Stirewalt, 1953). When the inter-exposure interval was one hour, a maximum average of 36 cercariae of the challenging exposure entered as compared with 45 from the same cercarial pool into previously unentered skin. Data for the 5 hour inter-exposure interval were 30 and 38 respectively;

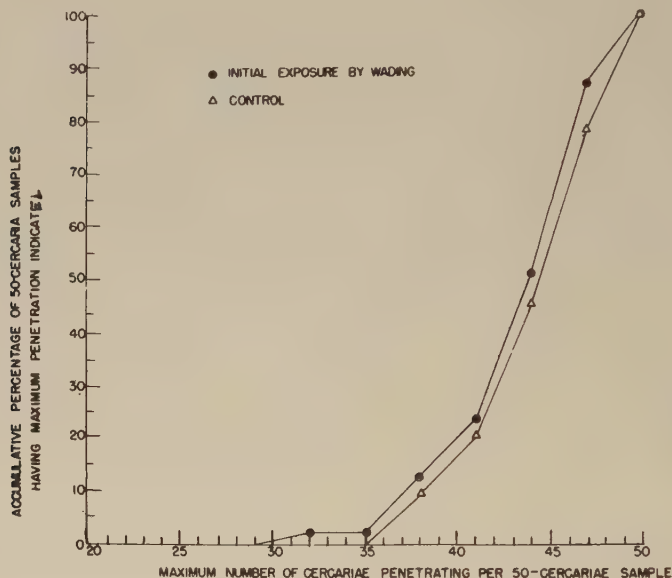


Fig. 4 — Accumulative frequency distribution showing percentage of 50-cercaria samples in which the maximum number penetrating was $\leq$ that indicated. Data are by class intervals of 3. (From Stirewalt, 1953)

for the 24 hour period, 36 and 43. Fig. 2 exemplifies graphically the accumulative frequency distributions of some of the same data.

Similar but more striking differences are recorded (Table 2) in the listing of the numbers of matured worms recovered at autopsy of the same mice. The average number of worms per mouse, labelled by their sex, was 7 from the challenging exposure compared with 20 from the initial exposure for the one hour inter-exposure interval; 9 against 18 for the 24 hour interval. Accumulated frequency distribution is shown in Fig. 3.

After 24 hours the inhibition diminished. The resistance was a local one as shown in Fig. 4, in which entries of challenging and initial cercariae were equal. For these data, the initial application of cercariae was to body skin, the challenging to tail skin.

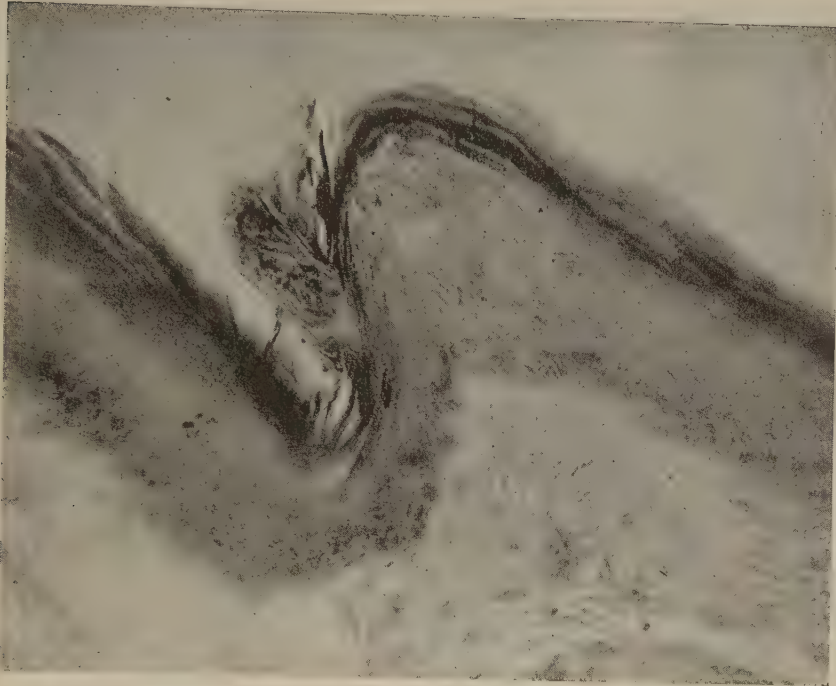


Fig. 5 — Entry into mouse tail skin at a follicular depression. There is corneal destruction and swelling around the parasite, but no extensive thickening. Biopsied five minutes after the application of cercariae. ($\times 375$)

To explain the mechanism of this local resistance, penetrated skin biopsied at intervals after exposure of from 5 minutes to 10 days was studied.

Within 5 minutes of the application of cercariae, the corneal layer was lysed (Fig. 5). The depth and appearance of this layer otherwise were normal. Within an hour or so of entry, the typical path of the schistosomulum lay just under the corneal layer. Extended

subcorneal movement was a characteristic pattern observed in mouse tail skin for several days. The corneal layer over this migration path became greatly thickened, often over an extensive area (Fig. 6). Beginning with the second and continuing through the fifth day

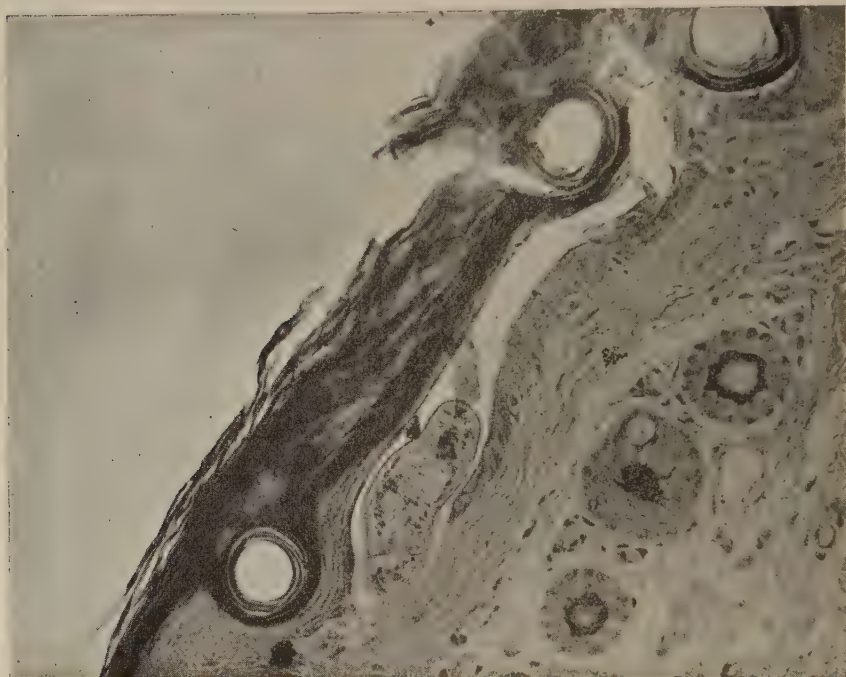


Fig. 6 — Migration path under the corneum of mouse tail skin, with a schistosomulum lying across the epidermis against the basement membrane. Corneal thickening shows above the migration path as compared with normal corneum in lower left. Biopsied one hour postexposure. ($\times 450$)

post-exposure, the corneal depth decreased as the thickened areas scaled off (Fig. 7). This scaling was observed macroscopically, as well as microscopically. By this time many schistosomula had moved into circumfollicular spaces (Fig. 7, lower center). The majority then moved into sebaceous glands. Little if any host cellular reaction was observed during this period. From this time on, the parasites moved deeper along follicular paths, or into dermal lymph spaces or follicular

venules, or transdermally into dermal or subdermal blood vessels. Some reversed direction and moved out toward the surface along paths similar to those followed inward. While movement was unimpeded little or no cellular reaction was produced. Wherever the parasites were stopped, however, leucocytes accumulated. Schistoso-

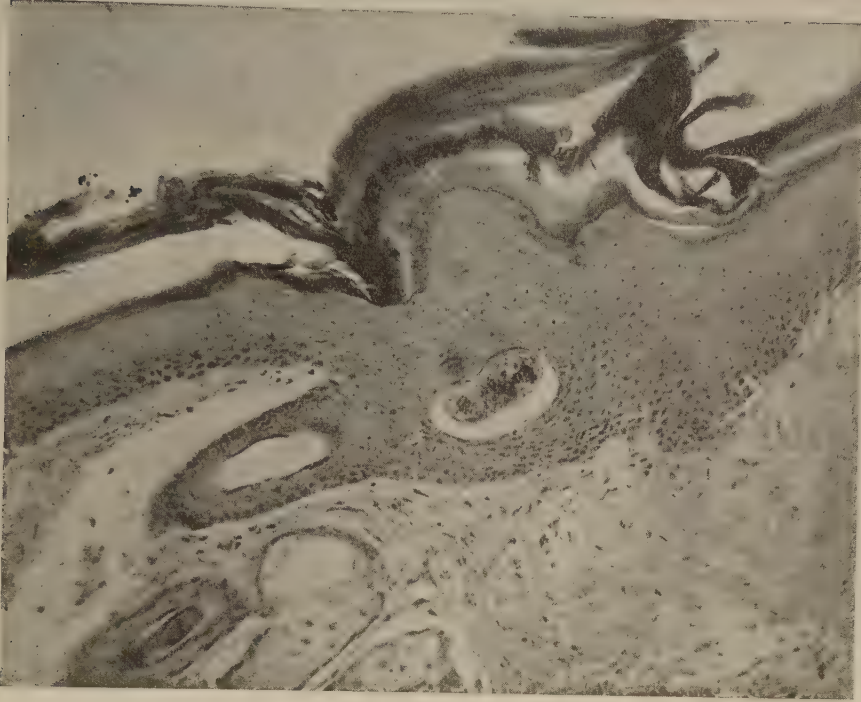


Fig. 7 — Corneal scaling in hyperkeratotic area. From the second through the fifth day postexposure. ($\times 185$)

mula were found surrounded by infiltrated host cells in both dermal and epidermal tissues.

Inhibition of entry was noted, however, only during that period between exposures when extensive areas of the *stratum corneum* were thickened, namely through 24 hours. It was therefore concluded that this thickening reduced the area of normal unthickened corneal tissue in recently penetrated skin as compared with unexposed skin,

and was thus responsible for the observed reduction in numbers of challenging parasites able to penetrate.

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SOME BIOPHYSICAL PROPERTIES OF AN ISOLATED CHR AND CERCARIAL AGGLUTINATING FACTOR FROM HUMAN ANTI-SERUM TO SCHISTOSOMIASIS MANSONI¹

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Evans, Stirewalt, and MacKenzie, in 1955, observed cercarial behavior in fractions of *Schistosoma mansoni* infected mouse sera obtained by moving-boundary electrophoresis. They suggested that the factors responsible for both cercaricidal activity and pericercarial envelope formation were contained in the gamma-globulin. It was further suggested that the «alpha-globulin» sample introduced a cercaricidal inhibitor which made it possible for the cercariae to survive and form envelopes. Envelopes were found, therefore, only in those infected serum samples which contained both gamma- and alpha-globulins or serum components with similar mobilities (Fig. 1).

Two basic difficulties, however, existed in interpreting data from samples obtained by the classical Tiselius fractionation method. They were (1) moving boundary fractionation did not give absolute separation of the serum components. Thus, all the components, except the slowest moving in the descending cell and the fastest in the ascending cell, are mixtures rather than electrophoretically pure, and (2) not only was marking exactly the boundaries between the components a

¹ The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

practical impossibility, but diffusion of the fractions during the time required for their removal from the cells, together with inevitable mixing of the liquid to some extent during removal introduced additional uncontrollable variables (Fig. 2).

The development of a continuous flow electrochromatography apparatus enabled us to overcome these difficulties and, in addition, lent advantage in that no two components were required to traverse the same path, and a reasonable number of subfractions with only slightly variant mobilities could be simultaneously recovered.

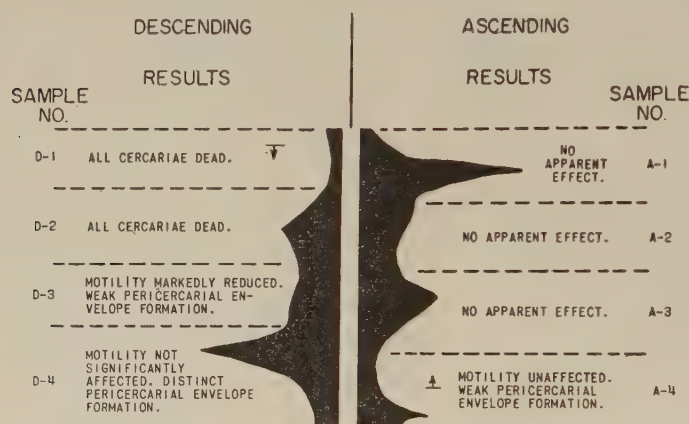


Fig. 1

MATERIALS AND METHODS

The infected serum used in the experiments reported here was from human cases of schistosomiasis mansoni of Puerto Rican origin. Serum samples from 16 cases on whom treatment records were not available for the first experiment and 12 untreated cases for the second were selected on the basis of CHR reactivity and pooled. As a control, sera from 20 consecutive admissions in a variety of disease states, but none with an animal parasite as a primary diagnosis, were obtained from the United States Naval Hospital, Bethesda, Maryland, and pooled.

Fig. 3. Electrochromatographic fractionation was carried out on a continuous flow paper electrophoresis system, essentially a modification of the apparatus described by Saroff (1955) and Adamik (1956).

In the first experiment, the major components were localized by biuret determinations on the material collected in each of the 28 take-off tubes. For the second experiment, the total pattern was determined

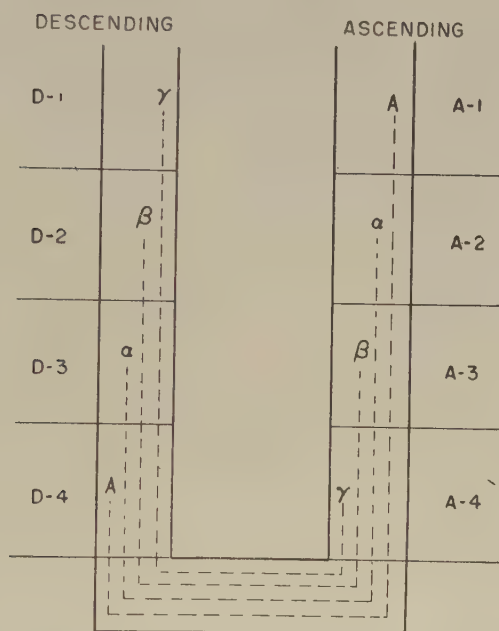


Fig. 2 — Composition diagram of sample components drawn from electrophoresis cell

in the same manner, but each tube was tested as an individual sub-fraction. In both experiments the test material was dialyzed to exhaustion against distilled water to remove the buffer, lyophilized, and reconstituted to 2 percent protein solution with physiological salt solution or triple-distilled water.

Electrophoretic homogeneity, sedimentation constants, chromatographic analyses, and *in vitro* tests for pericercarial envelope forming

and cercarial agglutinating activity of each fraction were carried out by standard methods.

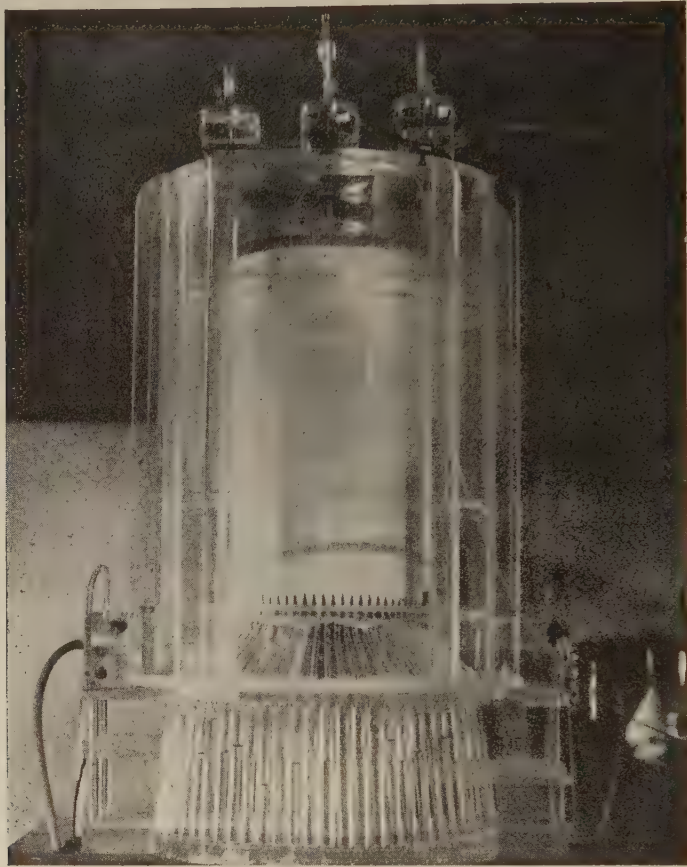


Fig. 3

RESULTS AND DISCUSSION

Fig. 4. In the first experiment, 6 major components were localized in each serum pool, infected and uninfected, and the lyophilized samples were combined as illustrated in this plot of the infected

serum. The broken line in the albumin area represents a tube to which the flow rate of electrolyte was inconstant.

Pericercarial envelope forming activity was found in only 2, fractions 5 and 6, of the 6 components of infected serum tested. However, there was a marked difference in the reactivity of these 2 components.

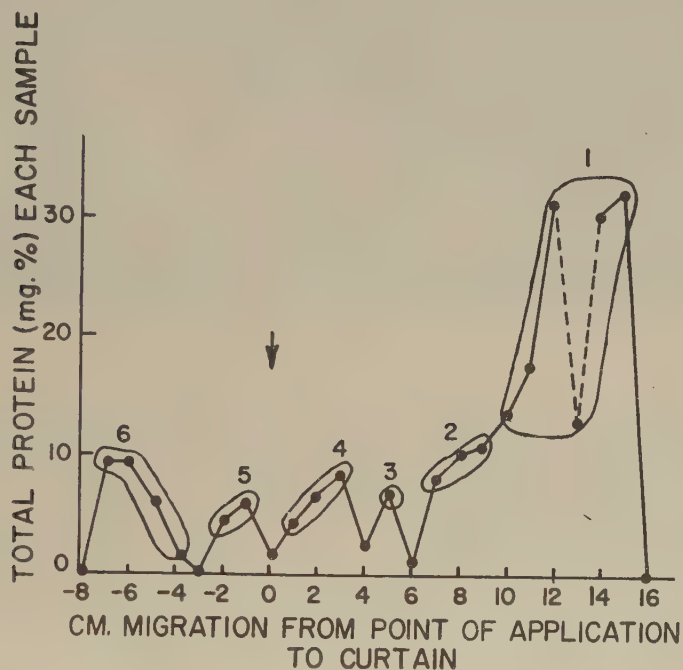


Fig. 4

Fig. 5. In fraction 6, by the end of the 3 hour observation period, well formed pericercarial envelopes had developed about the bodies of the cercariae.

Fig. 6. In fraction 5, however, while definitely reactive in this respect, the envelopes formed were minimal, smooth, and tight about the bodies of the larvae. The relationship between cercarial agglutination and pericercarial envelope formation has been shown previously by Stirewalt and Evans (1955); therefore, it was not sur-



Fig. 5



Fig. 6

prising that agglutination of the larvae occurred only in the same fractions which were positive for the CHR.

Fig. 7. It was noteworthy that, while electrophoretic analysis

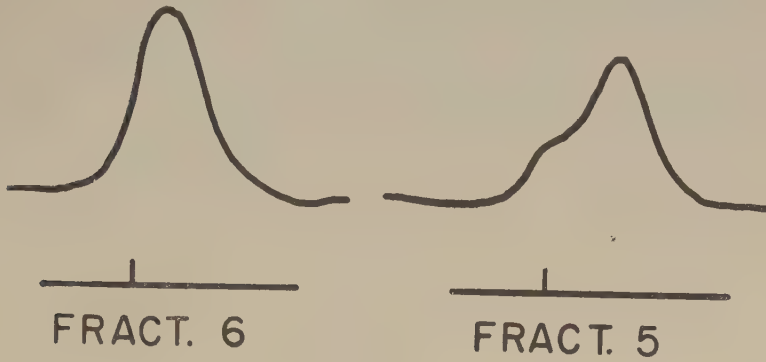


Fig. 7

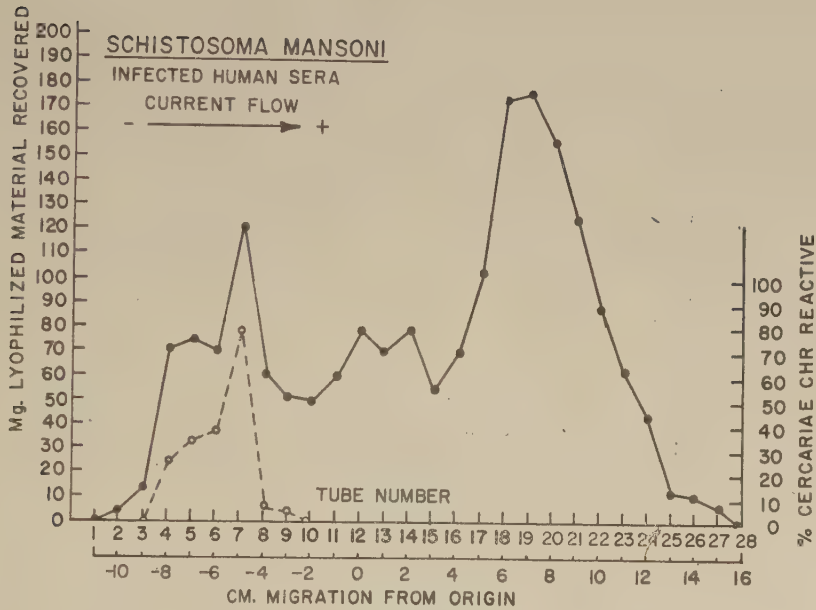


Fig. 8

of fraction 6 revealed only a protein with the mobility of human gamma-globulin, fraction 5 appeared to be a beta-globulin with some

contamination, probably by diffusion on the paper curtain, of a fast gamma-globulin. Assuming that the reactive serum component resided in the gamma-globulin alone, the difference in reactivity of the gamma- and beta-globulin components could be explained on the basis of the contamination of the latter which was present.

To test these results more rigorously, a second experiment in which the subfractions from each of the 28 tubes of the electrochromatography apparatus was tested individually was performed.

Fig. 8. In this case, the curve shown was obtained by plotting the actual weight of the recovered protein against the electrophoretic

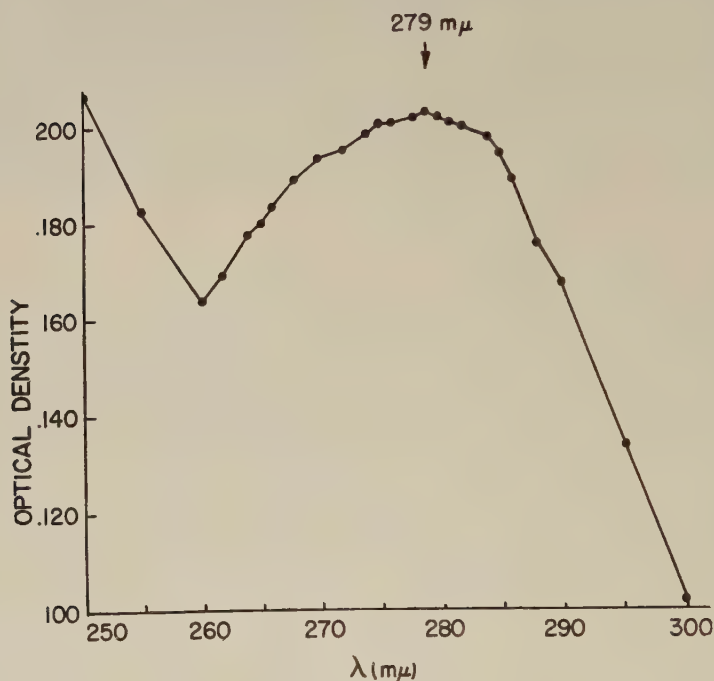


Fig. 9

mobility represented by the position of each tube. The broken line represents the pericercarial envelope forming activity of each cut.

It was immediately obvious that the pericercarial envelope forming activity was concentrated in tube number 7, and, since its mobility under the experimental conditions was toward the cathode,

what diffusion there was of the component in the paper curtain was principally in that direction.

A total of 121.0 mg of material was recovered from this cut of which 18.8 mg was soluble in triple-distilled water, and this water soluble portion was reactive with respect to the CHR.

Fig. 9. The absorption spectrum of this active material in the ultraviolet range revealed a maximum at 279 m μ , indicative of a tryptophane and/or tyrosine containing protein.

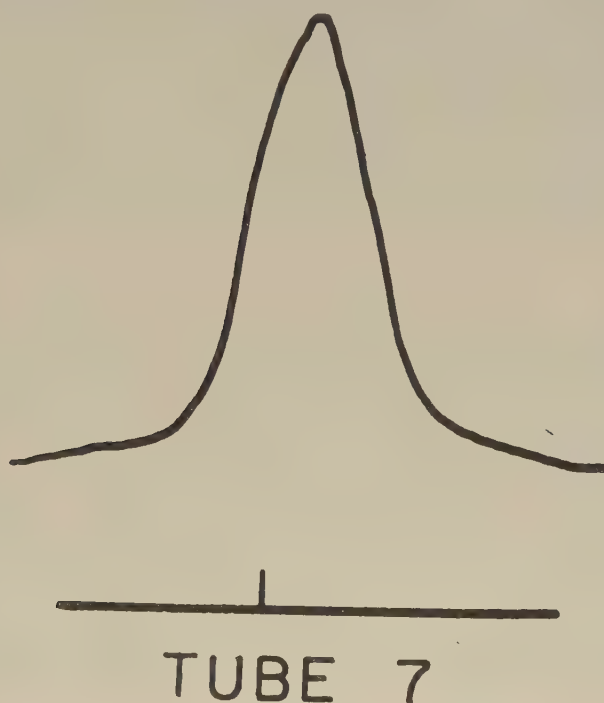


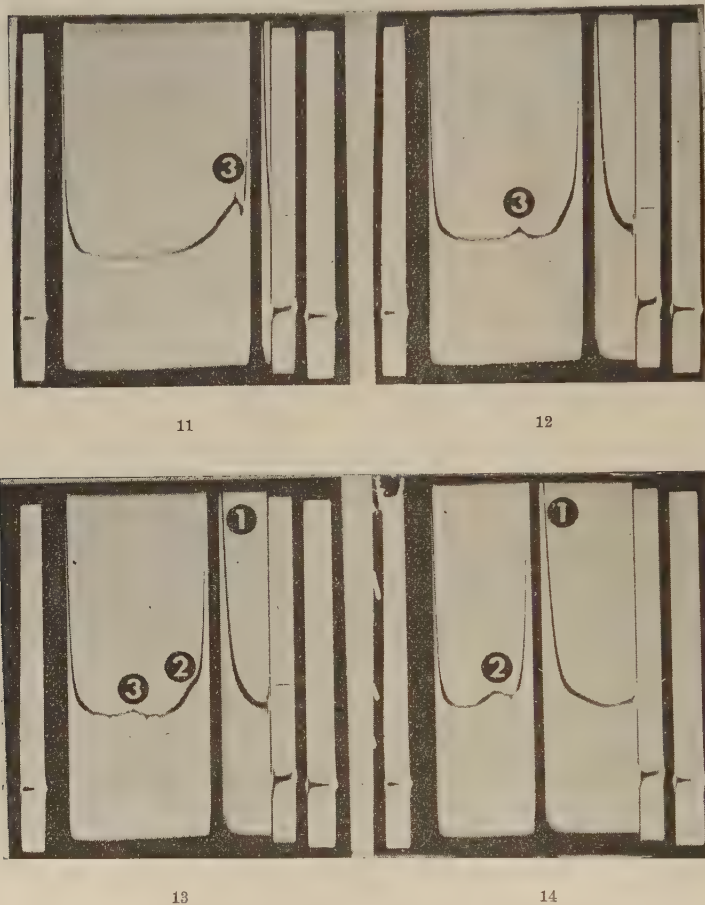
Fig. 10

Fig. 10. Paper electrophoretic analysis revealed that the active subfraction was an electrophoretically homogeneous gamma-1-globulin, or, as it is sometimes referred to, a T-globulin.

Qualitative ultracentrifugal analysis, however, revealed the fraction to be polydisperse, having two minor, comparatively fast-moving

fractions with sedimentation rates of 11.9 and 7.7 Svedberg units, respectively, and a major, slow-moving component with a sedimentation constant of 5.1 Svedbergs.

Figs. 11-12-13-14. These ultracentrifuge scannings illustrate



Figs. 11-14

the relative size and sedimentation rates of the 3 components. Unfortunately, the concentration was too high completely to resolve the large peak, but to lower the concentration sufficiently would have resulted in the loss of the smaller components. Pericercarial envelope

forming and cercarial agglutinating activities were isolated in this slow-moving major component.

The results of these experiments confirm and somewhat refine our previous suggestion that the antibody which manifests itself in vitro by the CHR is contained in the gamma-globulin fraction. As has also been found for a number of antibodies (Taliaferro, 1958), it is further localized in the faster moving gamma-1- or T-globulin portion as defined by electrophoretic mobility. One may assume from its slow sedimentation in the ultracentrifuge that the antibody molecule is of comparatively low molecular weight.

Table 1. Chromatographic analysis of the acid hydrolysates of the active subfraction revealed percentage of amino acid distribution

TABLE 1 — *Amino acid composition of acid hydrolysates of subfraction 7*

	Conc. %	Brand and Edsall, 1947		Conc. %	Brand and Edsall, 1947
Glycine.	4.8	4.2	Arginine	1.5	2.5
Alanine.	3.7	0.0	Histidine	8.4	8.1
Valine	9.0	9.7	Lysine	13.2	8.8
Leucine	5.5	12.0	Aspartic acid.	13.2	8.8
Isoleucine.			Glutamic acid.	15.6	11.8
Phenylalanine	4.1	4.6	Serine	11.4	11.4
Cysteine	2.6	0.7	Threonine	9.0	8.4
Methionine	0.2	1.1	Tyrosine	6.9	6.8

somewhat different from that assigned to the so-called normal human gamma-globulins by Brand and Edsall (1947). For example, the presence of 3.7 percent alanine in the antibody as contrasted to its reported absence in the so-called «normal» human gamma-globulins; the comparatively low concentration of leucine and isoleucine, and the comparatively high concentrations of cysteine, aspartic acid, and glutamic acid.

Obviously, no attempt can be made from these incomplete analyses to define the total differentiation of this isolated antibody from the remainder of the gamma-globulin complex. We have, however, we feel, a tool which may eventually enable us to define antibody structure at a molecular level without chemically denaturing its in vitro and perhaps even its in vivo activity.

SUMMARY

Sera of untreated human cases of schistosomiasis *mansoni* were fractionated by a continuous flow electrochromatographic technique. Six major components and a total of 28 subfractions were recovered. Each subfraction was tested in vitro for its active principle in terms of pericercarial envelope forming (CHR) and cercarial agglutinating activity. Both CHR and cercarial agglutinating activity were localized in a single subfraction which comprised about 0.6 percent of the total protein fractionated. The active principle isolated was soluble in triple-distilled water and was an electrophoretically homogeneous, fast-moving portion of the gamma-1-globulin (T-globulin) in 0.1 M veronal buffer, pH 8.6. Its absorption spectrum in the ultraviolet range revealed a single maximum at a wave-length of 279 m μ was suggestive of a tryptophane and/or tyrosine containing protein. In 0.15 ionic strength NaCl, qualitative ultracentrifugal analysis showed the fraction to be poly-disperse, having two minor, comparatively fast-moving fractions ($S_{20} = 11.9$ and 7.7, respectively) and a major, slow-moving component ($S_{20} = 5.1$). CHR and cercarial agglutinating activity were isolated in the major component. Chromatographic analysis of the acid hydrolysates revealed that the amino acid composition was somewhat different from that given for «normal» human gamma-globulin.

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2ND SESSION: Monday, September 8th—11:00 to 12:30 a.m. Room D

This session was opened by Dr. E. Carrol Faust, who asked Dr. F. Simões Barbosa to act as Chairman. The Rapporteur was Dr. F. S. Barbosa and the Secretary Dr. M. da Costa Faro. During this session the following papers were presented and at the end the general discussion was opened on the papers heard during the first and second sessions.

AVALIAÇÃO DOS RESULTADOS DA TERAPÊUTICA ESPECÍFICA DA ESQUISTOSSOMOSE MANSONI EM UMA CAMPANHA DE SAÚDE PÚBLICA NO BRASIL

O VALOR DAS DIFERENTES DROGAS E ESQUEMAS¹

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Embora a importância do tratamento específico do parasitismo humano pelo *Schistosoma mansoni* viesse a ser até certo ponto abalada, como método de valor, na erradicação da parasitose, com o recente reconhecimento de vários reservatórios extra-humanos do *S. mansoni* (1, 2), pelo menos no Brasil, é, entretanto, reconhecido o seu mérito como meio capaz de modificar o curso evolutivo, atenuando os processos lesionais da enfermidade, consoante as observações de S. Pachá no Egito (8) e de H. Sette em Catende (10),

¹ O autor expressa os seus agradecimentos ao Dr. Mário Pinotti, director-geral do Departamento Nacional de Endemias Rurais, do Ministério da Saúde, e ao Dr. Olimpio da Silva Pinto, coordenador da Campanha Nacional Contra a Esquistossomose, pela permissão para utilização dos dados deste trabalho, e bem assim aos que colaboraram nas diferentes tarefas relacionadas à sua elaboração: Dr.^a Maria Nazareth Hernandez Martim, encarregada do Posto de Touros, que também fez o levantamento da maioria dos dados aqui utilizados; Dr. João Cavalcanti de Albuquerque e Dr.^a Yeda Rabelo Baptista, respectivamente encarregados

no Brasil. Porém, como medida isolada, o tratamento específico em massa (7) não condiciona redução significativa dos índices de incidência da helmintose. Isto aconselha o tratamento, quer apenas de casos individuais, atendendo os doentes que possam evitar novos contactos infectantes, quer como medida colectiva, quando outros recursos, visando a erradicação da parasitose, possam ser postos em prática, tais como a luta antiplanorbídea, a execução de obras de saneamento ambiental e educação sanitária. Em tais condições, os resultados obtidos podem ser considerados dos mais satisfatórios, haja vista a redução do índice de infecção pelo *S. mansoni* em Varjão, estado de Paraíba, no Brasil, que de 53 por cento em 1954 passou para 20 por cento em 1957, apesar de realizadas apenas parcialmente as medidas de saneamento e antiplanorbídeas (6).

É bem verdade que certos aspectos ligados ao problema do tratamento específico constituem ainda uma incógnita, como a possibilidade de agravação da enfermidade, na decorrência da morte dos parasitas e da sua consequente migração para o fígado, onde ocorreriam lesões de certo vulto; a esse propósito, entretanto, é opinião da maioria dos pesquisadores brasileiros (4, 10, 12) que, respeitadas certas contra-indicações, a terapêutica específica, pelo contrário, resulta em tendência à melhoria, e não à agravação do quadro mórbido. Quanto aos riscos directos da quimioterapia, seu grau de eficiência, tolerância, custo e, enfim, praticabilidade da medicação esquistossomocida, cercados de certas limitações, serão considerados no corpo deste trabalho. Por certo não menos importante, porém menos conhecido, é o ângulo das repercussões da terapêutica específica sobre o estado imunitário das populações das áreas endêmicas, em contínua exposição às reinfecções, facto sobre o qual não se dispõe ainda de experiências (5, 11).

O presente estudo se refere a uma parte da casuística de esquistossomose mansonii submetida à terapêutica específica, pela Campanha Nacional Contra a Esquistossomose Mansonii no Brasil, no

dos Postos de Varjão e Mandacaru e de Cururupu. Também são credores de agradecimento os chefes das Circunscrições do DNERu de Paraíba, R. G. do Norte e Maranhão, respectivamente Drs. Otaviano Dustan Monteiro Filho, Osório Tenório Lima e Waldemar da Matta Pires, sob cuja administração funcionaram os postos de tratamento.

período de 1955 a 1957, na qual cerca de 25 000 doentes foram tratados por um dos esquemas adoptados, ainda em carácter não definitivo, e sobre os quais nos deteremos a seguir.

Tendo em vista a vantagem dos cursos menos intensivos, visando o tratamento ambulatorial, único viável em uma campanha sanitária (14, 12), optou-se pela adopção de esquemas semi-rápidos, utilizando os seguintes compostos antimoniais, que têm-se mostrado os mais vantajosos em nossa experiência: antimónio pirocatecol dissulfonato de sódio (APDS), gluconato trivalente de antimónio e sódio (GTAS) e antimónio dimercaptossuccinato de potássio (ADSP). Embora não definitivamente abandonado, o miracil D foi considerado impróprio para este tipo de campanha, dadas as manifestações de intolerância que o tornam muito impopular (12).

Não comportando as limitações de espaço deste trabalho descrição do critério selectivo dos casos submetidos ao tratamento, deve dizer-se, todavia, que apenas pacientes com forma hepatoesplênica avançada, com anemia pronunciada (hemoglobina abaixo de 60 por cento), além dos cardiorrenais e de idade acima de 50 anos, foram excluídos.

Dada a grande extensão da casuística e a precariedade das observações, mormente com relação ao *follow up* dos casos, cingir-se-á apenas à análise de uma parcela da mesma, que pôde ser acompanhada de modo considerado satisfatório, pois diversos obstáculos relacionados com o *contrôle* de cura impediram um acervo maior de observações a este respeito.

Analisaremos em primeiro plano o aspecto da tolerabilidade dos esquemas, aproveitando as casuísticas tratadas em Varjão e Mandacaru (Paraíba), Touros (R. G. do Norte) e Cururupu (Maranhão).

Tolerabilidade. — Figuram no quadro 1 os dados referentes a este aspecto; para se estabelecer um cotejo dos medicamentos ensaiados deve-se atentar para três factos principais:

1) A posologia total utilizada, em teor de Sb III por quilo de peso, que foi de 8,5 mg para o APDS, de 10,8 mg para o ADSP e de 5,5 para o GTAS;

2) A duração do curso terapêutico, mediante injeções diárias, previstas para 12 dias com o primeiro composto e para 6 dias com os dois outros;

3) A via de introdução do medicamento, que foi a intramuscular para os dois primeiros mencionados e a intravenosa para o último.

Tacteando a sensibilidade dos doentes, procurou-se administrar o esquema dentro de um prazo o mais aproximado possível do previsto, mediante a injeção de fracções iguais, diariamente. Algumas modificações no espaçamento das doses não puderam, entretanto, deixar de ser feitas — ora por causa de intolerância, ora por outras razões —, as quais não foram convenientemente avaliadas. Como, porém, estas últimas devem ter ocorrido de maneira aproximadamente igual com os diversos medicamentos, pelo menos em cada posto de tratamento, sob os cuidados de um mesmo médico, poderemos aceitar as cifras médias do número de dias para a efectivação de cada esquema como um bom índice da tolerabilidade do tratamento com cada uma das substâncias.

Uma análise dos dados do quadro 1 evidencia variações no número médio de dias de realização do esquema, não apenas com referência a cada uma das substâncias, mas também entre estas, conforme a localidade. Tal facto ocorreu também com respeito às *incidências das manifestações e das interrupções forçadas*, estas últimas dependentes em parte das primeiras, mas também de outros factores, entre os quais a *intensidade das manifestações*. Este último factor, de mais difícil apreciação, pode ser aferido em parte pelos dados do quadro 2, referente à natureza das *manifestações de intolerância*, que nunca foram responsáveis por acidentes graves, pois apenas uma morte foi registada entre o total de 25 000 casos, atribuível a uma omissão, visto ter ocorrido em caso com contra-indicação formal à estibioterapia ambulatorial, representada por cardiopatia hipertensiva.

A importância de um factor local, na expressão das variações assinaladas, parece indiscutível, haja vista o que se observou em Touros, onde a incidência das manifestações de intolerância foi a menor de todas para cada substância, resultando em um menor percentual de interrupções forçadas e na execução do esquema em um período mais próximo do previsto. Esta variabilidade pode ser atribuível ao estado geral dos grupos populacionais, possivelmente subordinado a condições carenciais e a associação a diferentes verminoses intestinais. Estes factores, embora operando em todas as áreas endémicas, pareciam ser menos intensos em Touros. Outro factor de variação decorreria do critério pessoal no modo de distribuir a posologia

QUADRO 1 — Tolerabilidade aos antimoniais

Droga	Via de administração	Posologia por quilo de peso		Localidade	Número inicial de doentes submetidos ao esquema	Manifestações de intolerância		Interrupções do esquema				Número final de doentes que completaram o esquema	Número médio de dias para administração do esquema
		Do composto	Em Sb III			Número de doentes	Porcentagem	Número de doentes	Porcentagem	Número de doentes	Porcentagem		
Antimónio piro-catecol dissulfato de sódio (APDS)	Intramuscular	1 ml Previsto para 12 dias	8,5 mg	Paraíba (Varição e Mandacaru) R. G. do Norte (Touros) Maranhão (Cururu)	703	298	42,3	67	9,5	36	5,1	600	22,2
						17	9,1	10	5,7	1	0,57	164	15,6
Antimónio dimercaptossuccinato de potássio (ADSP)	Intramuscular	40 mg Previsto para 6 dias	10,88 mg	Paraíba (Varição e Mandacaru) R. G. do Norte (Touros) Maranhão (Cururu)	51	35	68,6	2	3,9	9	7,6	40	10,4
						7	22,5	1	3,2	2	6,4	28	7,0
Gluconato trivalente de antimónio e sódio (GTAS)	Intravenosa	17 mg Previsto para 6 dias	mg 5,56	Paraíba (Varição e Mandacaru) R. G. do Norte (Touros)	43	26	60,4	0	0	5	11,6	38	11,5
						33	27,7	0	0	3	2,5	116	7,9

em face das reacções de intolerância e da diversidade na extensão das tarefas de cada médico, que foi sempre o mesmo para cada posto.

Da análise feita salienta-se a melhor tolerabilidade do APDS, tendo em vista o conjunto de requisitos considerados, seguido do ADSP e do GTAS. Entretanto, dada a intensidade do tratamento, os dois últimos apresentam vantagens, pelo encurtamento do curso, mormente considerando-se, de um lado, a elevada posologia do ADSP e, de outro, a via intravenosa, no caso do GTAS.

Uma apreciação final permite concluir-se pela necessidade de se adaptarem os diferentes esquemas aos níveis de tolerabilidade indi-

QUADRO 2 — *Tipo das manifestações de intolerância*

Tipo de manifestação	Medicamento		
	APDS (600 casos)	ADSP (150 casos)	GTAS (150 casos)
Náuseas e/ou vômitos. . .	10 0/0	26,6 0/0	16 0/0
Hipertermia	1 »	8 »	6,6 »
Dores musculares	5 »	2,5 »	9,3 »
Cefaleia	6 »	10,6 »	10,6 »
Dores abdominais	3 »	5,3 »	3 »
Artralgia	3 »	15,3 »	5,3 »
Prurido cutâneo	1 »	4 »	0,66 »
Lipotimia	0 »	1,3 »	6 »
Tonturas	2,3 »	7,3 »	3 »
Diarreia	0,66 »	4 »	0,66 »
Tosse.	0	0	0,66 »

vidual, por vezes expressão de condições colectivas, nos diferentes agrupamentos humanos. Não parece ser vantajoso ou mesmo possível o aumento da posologia total, excepto no caso do GTAS, embora possa presumir-se que um encurtamento do curso terapêutico pelo APDS seja viável. Nesse sentido, já foram estabelecidas normas, visando a adopção de um esquema de 20 mg a 24 mg por quilo de peso com o GTAS, e para a aplicação do curso de APDS em um período de 6 a 8 dias. Ressalte-se que várias medidas tiveram de ser adoptadas, no sentido de uma melhor tolerância à droga, subsequentemente às primeiras manifestações de intolerância, mediante o uso de sedativos, antiespasmódicos, antialérgicos e outros correctivos.

Outro ângulo do problema, que parece de importância primacial, é o que concerne a diferenças na tolerabilidade dos diversos grupos etários; este aspecto, que é pormenorizado no quadro 3, evidencia uma menor frequência de manifestações de intolerância nas idades mais baixas, principalmente antes dos 16 anos.

Outros aspectos práticos. — Do ponto de vista económico, embora o custo da droga seja importante, e neste caso o APDS é o mais vantajoso, o consumo de trabalho e de tempo para a sua aplicação pode não oferecer compensações; enfim, sob outro ângulo de pra-

QUADRO 3 — *Tolerabilidade em função dos grupos etários*

Grupo etário	Percentagem de reacções por medicamento		
	APDS (494 casos)	ADSP (123 casos)	GTAS (165 casos)
3-10 anos	12	33,3	33,3
11-15 anos	12,1	28,5	17,1
16-20 anos	21,4	30,8	36,3
21-30 anos	61,7	71,8	41,6
31-40 anos	53,7	61,9	43,2
41-50 anos	46,5	50,0	52,0
<i>Global</i>	<i>30,1</i>	<i>48,7</i>	<i>36,8</i>

ticabilidade, ressalta-se a vantagem do APDS e do ADSP, dada a sua possibilidade de administração intramuscular, ao que acresce, no caso do primeiro, a grande estabilidade das soluções prontas para uso.

Eficiência terapêutica. — É este aspecto do problema de apreciação mais complexa, dados certos factos obscuros com ele relacionados, mormente as dificuldades de *contrôle* da reinfecção nas áreas endémicas da parasitose. Estas foram apenas parcialmente, e em um grau variável, contornadas. Como praxe, a instituição da terapêutica específica em uma determinada área só se iniciava após 2 meses do combate antiplanorbídeo, feito principalmente nos focos peridomiciliares. Esta medida visou reduzir a possibilidade de infecções não amadurecidas, sabidamente invulneráveis à acção da terapêutica específica.

As possibilidades de reinfecção permaneceram reduzidas com o prosseguimento da aplicação de planorbicidas e com o trabalho de educação sanitária.

Adoptou-se como critério de eficiência terapêutica a negativação coproscópica, pelo método de sedimentação de Hoffman-Pons e Jenner, em 4 amostras colhidas em dias diferentes, a partir do 4.º mês do término do tratamento e nunca excedendo do 6.º mês. É certa a precariedade deste critério, que seguramente poderia ser melhorado mediante: a) realização de maior número de exames em cada amostra; b) utilização de maior número de amostras; e c) prolongamento do tempo de observação. O último factor, entretanto, pode tornar-se responsável pelo falseamento dos resultados, visto concorrer para uma maior possibilidade de reinfecção. Segundo os trabalhos experimentais de Vogel e Minning (13) e de Prata (9), a propósito das alterações do ciclo de postura dos esquistossomos das espécies *japonicum* e *mansoni*, após a estibioterapia, parece-nos suficiente o *contrôle* a partir do 3.º mês e, com maior margem de garantia, a partir do 4.º mês (12). Quanto ao número de exames, consoante o trabalho de Brener e Chiari (3), as 4 pesquisas fornecerão informes válidos da ordem dos 76 por cento. Na impossibilidade de complementação desse critério, através da biopsia rectal, em uma campanha sanitária, consideramo-lo suficiente, até que surjam meios mais eficientes e práticos aplicáveis a esse tipo de trabalho.

Uma análise dos dados do quadro 4 permite uma apreciação panorâmica da eficiência terapêutica obtida com as três drogas ensaiadas, computados os resultados nos casos que receberam a dose total, apenas com variação para menos, até o máximo de 10 por cento.

Considerando-se apenas os casos submetidos ao *contrôle* de 4 exames, o que somente foi observado na casuística de Paraíba, verificamos os índices de negativação de 80,3 por cento com o APDS, de 81 por cento com o ADSP e de 86,5 por cento com o GTAS. Embora os dois últimos, lamentavelmente, não figurem em casuísticas numericamente mais significativas, evidenciam resultados bastante aproximados dos obtidos com o ADPS, acrescida neles a vantagem de realização em um prazo mais curto, apesar de uma maior incidência de reacções e mesmo de interrupções forçadas.

Outro facto interessante é o que advém da análise dos resultados obtidos, tendo-se em conta os diferentes grupos etários; obser-

QUADRO 4 — *Eficiência terapêutica*

Medicamento	Localidade	Negativação parasitológica													
		Por grupos etários													
		3-10 anos		11-15 anos		16-20 anos		21-30 anos		31-40 anos		41-50 anos		Global	
		Número de casos	Perccntagem	Número de casos	Perccntagem	Número de casos	Perccntagem	Número de casos	Perccntagem	Número de casos	Perccntagem	Número de casos	Perccntagem	Número de casos	Perccntagem
		Neg.	Tot.	Neg.	Tot.	Neg.	Tot.	Neg.	Tot.	Neg.	Tot.	Neg.	Tot.	Neg.	Tot.
APDS 1 ml por quilo de peso	Paraíba (Mandacaru e Varião)	51	85	55	87	66	69	96	112	102	105	56	62	426	530
	R. G. do Norte (Touros)	15	33	16	24	7	10	1	1	3	3	3	3	45	74
	Maranhão (Cururupu)	1	4	5	6	10	11	5	7	6	7	5	6	32	41
	Minas Gerais (Araxá)	0	2	0	23	0	6	0	2	1	1	0	0	1	34
ADSP 40 mg por quilo de peso	Paraíba	1	3	5	6	2	4	5	5	3	3	—	—	17	21
	Maranhão	4	4	8	9	5	5	10	10	7	7	8	8	42	43
GTAS 17 mg por quilo de peso	Paraíba	—	—	1	2	6	6	3	4	4	5	5	5	19	22
	R. G. do Norte	2	4	9	11	9	12	8	12	9	9	13	14	50	62

va-se, com os três medicamentos, de modo muito constante, um maior percentual de negatificação nos grupos de idade mais elevada, de maneira geral após os 16 anos, comparados aos de mais baixa idade. Uma apreciação sobre as causas dessas variações, de ordem especulativa apenas, à base dos dados disponíveis, permite sejam levantadas as seguintes hipóteses:

1) Maior resistência dos parasitos nos grupos etários mais baixos, por serem de vermes mais jovens, biologicamente menos vulneráveis à estibioterapia, pelo menos no que toca à sua função genital; isto porque o método de análise baseia-se na presença ou ausência dos ovos viáveis do parasito;

2) Maior actividade metabólica do organismo humano abaixo dos 16 anos, com consequente maior inactivação do medicamento, disso decorrendo menor actividade esquistossomida;

3) Maior possibilidade de reinfecção nos grupos etários mais baixos, dadas a sua menor compreensão do problema e outras dificuldades de *contrôle*, que aumentam as possibilidades de exposição, ou ainda uma sua maior susceptibilidade, devida a um grau imunitário mais baixo do que o do adulto. Também factores profissionais, paralelos aos grupos etários e variáveis com as localidades, poderiam estar em jogo.

Possivelmente, uma interacção de mecanismos deve actuar no determinismo dessas variações, o que aconselharia, do ponto de vista terapêutico, o aumento das doses medicamentosas nos grupos etários mais baixos. Tal conduta se justifica pela melhor tolerabilidade dos mesmos, conforme foi assinalado; no caso corrente deve ser olhada, entretanto, com maiores cautelas, pois os riscos da antimonoterapia correm parilhas com as dificuldades de *contrôle* dos pacientes, principalmente o repouso físico — de mais difícil obtenção nesses grupos etários.

Que um outro mecanismo parece interferir nos resultados da terapêutica específica, sugere-o o facto de também se observarem variações nos resultados, de acordo com a área estudada. Para uma melhor apreciação desse aspecto, incluímos no quadro 4 os resultados obtidos em outra localidade, a cidade de Araxá, em Minas Gerais.

Utilizando em outras áreas o APDS, com o mesmo esquema de Paraíba, embora, lamentavelmente, controlado apenas por uma coprocopia, são bastante curiosos os resultados, mostrando uma nítida infe-

rioridade no percentual de negativas, que foi de 79,1 por cento em Cururupu, 60,9 por cento em Touros e 2,94 por cento apenas na cidade de Araxá. Considerando-se os resultados de acordo com os diferentes grupos etários, mantiveram-se as diferenças assinaladas na casuística de Paraíba, com algumas modificações que não podem ser levadas em grande consideração, raciocínio aliás aplicado aos outros aspectos, pela menor expressão numérica das casuísticas. Chamam, no entanto, a atenção, de modo gritante, os resultados observados em Araxá, onde impressiona o coeficiente nulo de negativas, ao primeiro reexame, representado por um único caso entre 34. Curiosamente, o ADSP apresentou em Cururupu resultados bastante mais satisfatórios, calcados em idêntico critério de apreciação de cura, superando os registados com as outras duas substâncias, nos casos submetidos ao mesmo critério. Ressalte-se, entretanto, a insuficiência do valor desse *contrôle*.

O factor ou factores responsáveis por essas diferenças de comportamento não podem ser apurados com facilidade, exigindo investigações que estão em curso, entre as quais as seguintes merecem consideração:

1) *Variações quanto à possibilidade de reinfecção*. Neste particular, nas localidades de Paraíba ocorreram melhores condições de *contrôle*, principalmente porque aí se desenvolveu um trabalho de educação da comunidade. Quanto à relação com os índices locais de incidência da helmintose, paradoxalmente, Araxá, que figura com os mais baixos de todos, parece ter sido mais propícia à reinfecção;

2) *Variações quanto ao nível imunitário*. Com respeito a este aspecto, as localidades de Paraíba apresentariam nível mais elevado, por se tratar de focos antigos, enquanto os demais, principalmente Araxá, de recentíssima implantação, pressuporiam menor grau imunitário da população;

3) *Variações quanto à raça do parasito*;

4) *Variações quanto a problemas estritamente ecológicos*. Deste ponto de vista, é curioso assinalar-se que Araxá está situada no altiplano do Brasil Central, enquanto as outras localidades situam-se no litoral do Norte e Nordeste. Ocorre ainda que aquela apresenta condições peculiares, decorrentes de fontes de águas radioactivas e sulfurosas.

Fica, apenas em carácter especulativo, levantada a hipótese da interferência de factores como o iodo, o enxofre e a radioactividade.

O último poderia actuar sobre o aspecto imunitário, reduzindo a anticorpo-gênese. O primeiro, sobre funções metabólicas de reconhecida importância, com repercussão não somente sobre o ângulo imunitário, como em outras esferas; e, finalmente, o enxofre, reduzindo a acção esquistossomicida dos antimoniais, por meio de grupos sulfidríla.

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CLINICO-PATHOLOGICAL, HOEMODYNAMIC STUDIES AND MANAGEMENT OF HEPATO-SPLENIC SCHISTO- SOMIASIS IN EGYPT

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Hepatic schistosomiasis in Egypt is mostly due to mansoni infection complicated by different grades of parenchymal and extra-hepatic vascular complications, for long considered as one clinical entity called «Egyptian splenomegaly».

The variability in the clinical manifestations, laboratory investigations of this disease as well as the variable response to medical or surgical management, encouraged to establish a team of workers to study the physiopathology of its various forms by the established methods of investigations recently applied in studies of hepatic disorders. A series of 180 cases were first studied biochemically for liver functions and histologically by liver biopsy.

A second series of 46 cases were also subjected to a thorough clinico-pathological, radiological and hoemodynamic study. Their por-

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tal pressures were measured by the intra-splenic and occluded hepatic vein methods. Right auricular and free hepatic vein pressures were also measured to exclude post-hepatic causes of portal hypertension. Direct portal vein pressure was estimated in cases submitted to surgical management through one of the omental tributaries. Increased intra-abdominal pressure by compressing the anterior abdominal wall or by pneumoperitoneum had been utilized in 20 cases to find out whether or not such an increase would be reflected on the intra-splenic and occluded hepatic vein pressures. Pneumoperitoneum had also been utilized in some cases to visualize perisplenic adhesions pre-operatively. A battery of liver function tests was utilized in both series as follows:

- A — *The parenchymal unit*, including:
 - 1 — The synthetic activities of liver:
 - a) Albumin content of serum.
 - b) Hippuric acid synthesis.
 - c) Prothrombin synthesis.
 - 2 — The carbohydrate functions of liver.
- B — *The colangiolar unit*:
 - 1 — Icterus index.
 - 2 — Bilirubin in blood.
 - 3 — Alkaline phosphatase.
- C — *The vascular unit*:
 - 1 — Bromsulphthalein test.
- D — *The reticulo-endothelial unit*:
 - 1 — Flocculation tests.

Portal circulation time had been estimated in the second series by using 10-20 mg lobeline and subtracting the hepatic-carotid circulation time from that of the spleno-carotid.

Lieno-portography was applied to the latter series to visualize the portal system and to diagnose any extra-hepatic thrombosis.

Radioscopy of the oesophagus for evidence of varices was also utilized. Some cases were studied histologically by wedge biopsy during laparotomy.

The above methods of investigations made possible the adoption of a new scheme of classification based on a dynamic and functional

approach rather than on the relative sizes of liver and spleen as used before:

- I — The uncomplicated bilharzial group.
- II — The complicated parenchymo-vascular (mixed group):
 - i) Compensated.
 - ii) Decompensated.
- III — The complicated pre-hepatic vascular group:
 - i) Portal vein thrombosis.
 - ii) Splenic vein thrombosis.
- IV — The inactive non-progressive group.

I — THE UNCOMPLICATED BILHARZIAL GROUP (26 percent of the series).

The specific histopathology has been shown to be different from all other types of cirrhosis. The peripheral eosinophilic granuloma, leading to interstitial fibroblastic reaction, which extends gradually in a tapering fashion to enclose several lobes, completely or incompletely, giving a peculiar polygonal pattern with minimal parenchymal involvement. Puffiness of face, oedema of upper half of body, jaundice palmar erythema and gynecomastia are unusual findings in this group and cholemia was very late.

The spleen was enlarged to a variable extent in half the cases while the liver was firm in consistency and both lobes were equally enlarged. The anterior abdominal veins showed dilated collaterals in one third of the cases.

The liver function tests showed the following features:

- 1 — Very late implication of the synthetic function of the liver.
- 2 — Very late derangement of carbohydrate metabolism.
- 3 — B.S.P. retention in 35 percent of the cases.
- 4 — Mild biliary implication most marked in alkaline phosphatase levels.
- 5 — Special pattern of dysproteinoemia characterized by marked rise of globulins.
- 6 — All flocculation tests were positive in a high percentage of cases, especially takata ara.

Portal manometry: only 5 percent of the cases showed levels below 20 cm of saline, indicating early stages of the disease. The intra-splenic pressure was highly raised in the rest of the cases, while the occluded hepatic vein pressures showed much lower figures indicating a pre-sinusoidal obstruction which is different from that of portal cirrhosis.

Portal venography (Figs. 1, 2, 3 and 4): demonstrated a patent widely dilated and tortuous splenic and portal veins, the intra-hepatic tributaries were variably withered and non-visualized in long standing cases. Visible collaterals were easily demonstrated and the lack of filling of the left portal vein in some cases explained the absence of dilated abdominal veins due to obliteration of the para-umbilical veins.

Portal circulation time: was found prolonged in some cases and shorter in others according to degree of established collaterals. In one case a double response was noticed.

Management: general and specific therapy were applied cautiously in cases with active infestation with moderate portal hypertension and not in grossly hypertensive cases.

Splenectomy: though it lowered the portal tension temporarily and immaterially, yet it has been advised whenever there was evidence of hyper-splenism unless the spleen was adherent when splenic artery ligation was advisable.

Spleno-renal shunt: was advised in absence of hoematemeses in cases with gross portal hypertension and markedly dilated oesophageal varices.

II — THE COMPLICATED PARENCHYMO-VASCULAR (MIXED GROUP) (61 percent of the series).

These cases showed, besides histological evidence of bilharziasis, manifestations of nutritional or post-hepatic cirrhosis. In advanced bilharzial cases the liver cells are affected lastly by ischaemic atrophy.

The following clinical features were noticed in this group:

- 1 — History or present evidence of jaundice.
- 2 — High incidence of residual or recurrent symptoms of pellagra,

anaemia, diarrhoea, endemic parotitis, other signs of avitaminosis or relatively increased endocrinal imbalances.

- 3 — Lower levels of portal tension with less collaterals, relatively smaller spleen and unequally affected nodular lobes of liver.
- 4 — Higher incidence of hypo-proteinaemia and rapidly filling ascites. — 17 percent of the series were compensated and 44 percent showed evidence of decompensation whether inform of ascites, choloemia or hoematemesis.

Hoematemesis in these cases though common is rarely fatal (if properly controlled) though rapidly followed by ascites. Predisposing factors of hypo-proteinaemia were always present for some time before appearance of ascites. Hypo-prothrombinaemia is not marked in our series of cases.

The liver function tests showed variable degrees of encroachment on the parenchymal and cholangiolar functions in addition to the vascular and reticulo-endothelial ones — evidence of hypersplenism was found to go hand in hand with the degree of hepatic dysfunction.

Portal venography (Figs. 5, 6 and 7), circulation time and portal manometry helped in indicating the degree of vascular involvement in each case and showed less discrepancy between the splenic and occluded hepatic pressures.

Management: it is mainly medical rather than surgical; specific therapy is contra-indicated.

Splenectomy, Tanner's operation and hepatic artery ligation may be resorted to in cases with repeated hoematemesis.

III — THE COMPLICATED PRE-HEPATIC VASCULAR GROUP.

Vascular thrombosis of the splenic, portal veins or both were met with in 13 percent of the cases. It modified the clinical picture, haemodynamics and venograms of such cases as well as their surgical management.

Besides the gross enlargement of the spleen in proportion to the cirrhotic process of the liver, hoematemesis secondary to rupture of a dilated oesophageal varix were liable to occur.

Anterior abdominal wall veins were not dilated and venous hum in the epigastrium, always lacking as the obstruction is pre-hepatic

ascitis, was usually lacking. Unless the thrombotic process has extended to the extra-hepatic tributaries.

Portal manometry: revealed a marked exaggeration of the already present discrepancy between the intra-splenic and occluded hepatic pressures denoting an extra-hepatic obstruction demonstrated more by abdominal compression or pneumo-peritoneum.

Portal venography (Figs. 8 and 9): it was able to demonstrate the site and extent of the thrombotic process; one or more of the hilar splenic venules may be thrombosed, showing filling defects, while on others the main splenic or portal veins may be occluded with no contrast material reaching the liver. The collateral veins are hugely dilated according to the site of the occlusion.

Delayed splenic emptying is helpful in the diagnosis of portal occlusion.

Liver function tests: were helpful to show the state of liver parenchyma and to guide any advised surgical interference.

Portal circulation time: was prolonged in most of the cases, except in long standing cases with grossly dilated collaterals.

Management:

1 — Splenectomy with coronary vasoligation was indicated for cases of splenic vein thrombosis unless the spleen was adherent when splenic artery ligation is applied.

2 — Leino-renal anastomosis was restored to in few cases of portal vein thrombosis, but the mortality is high and it is preferable to leave them alone.

N.B.: Porto-caval anastomosis is completely contra-indicated in such cases.

IV — THE INACTIVE NON-PROGRESSIVE GROUP.

Where the reticulo-endothelial evidence of activity of the bilharzial infestation or the parenchymal evidence of the nutritional or the post-viral cirrhotic process became stationary, the prognosis is different. They do not need any further interference as they proved quiescent after a long follow up provided that these patients had changed their residence or occupations to obviate any further exposure to infection.

SUMMARY AND CONCLUSIONS

1—The new methods of investigation in hepato-splenic disorders, like intra-splenic and occluded hepatic vein pressure estimations with or without compression, portal venography and portal circulation time detection, have been utilized in the investigation of our endemic bilharzial hepato-splenic disease.

2—A series of this syndrome have been submitted to the different methods of investigation by the staff of the Kasr-El-Aini Hepatic Research Unit. All the cases have been examined clinically, haematologically, biochemically, as well as by liver biopsy and some were further subjected to portal manometry and portal venography. It was possible after following the preceding scheme to discriminate any given case relating it to its original group given in the new classification of the syndrome.

3—Certain clinical findings encountered in the course of that syndrome, like the presence or absence of dilated abdominal wall veins, haematemesis and endocrinal dysfunctions, have been discussed and correlated to the underlying physiopathological status of the different groups of that syndrome.

4—The liver function tests proved to be of much diagnostic value in differentiating the different subgroups of that syndrome whether they were purely bilharzial, or mixed with parenchymal involvement.

5—Portal manometry estimations in the uncomplicated bilharzial cases showed a distinct pattern which may be diagnostic in most cases, and which was found different from that found in portal cirrhosis.

6—Incidence of extra-hepatic vascular complications in this syndrome diagnosed ante-mortem had been stressed.

7—Abdominal compression or the application of pneumo-peritoneum to raise the intra-abdominal pressure could help in confirming the diagnosis of extra-hepatic portal vein occlusion.

8—Management of each group had been standardized according its pathogenesis and according to the clinical results, obtained medical or surgical procedures.

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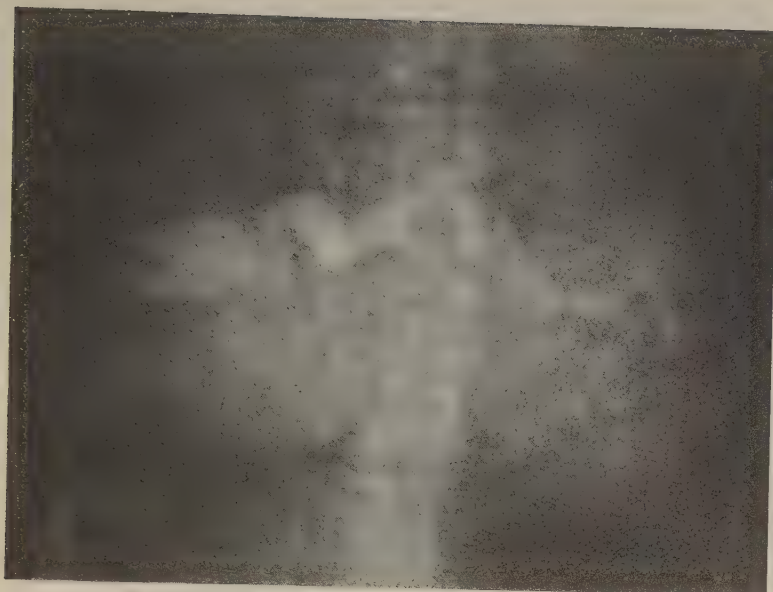


Fig. 1 — Normotensive pure bilharzial

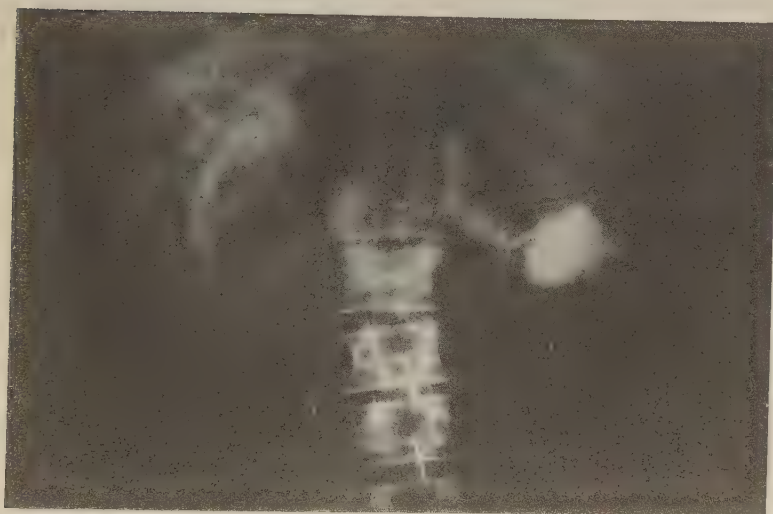


Fig. 2 — Mild hypertensive pure bilharzial

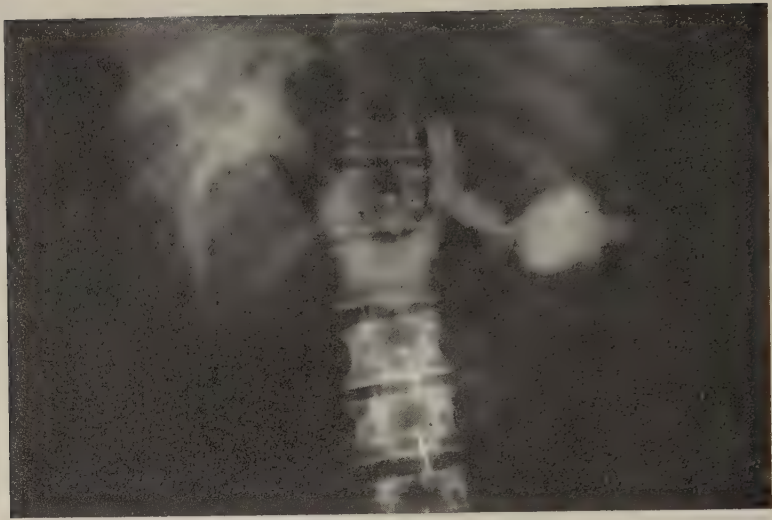


Fig. 3 — Moderate hypertensive pure bilharzial

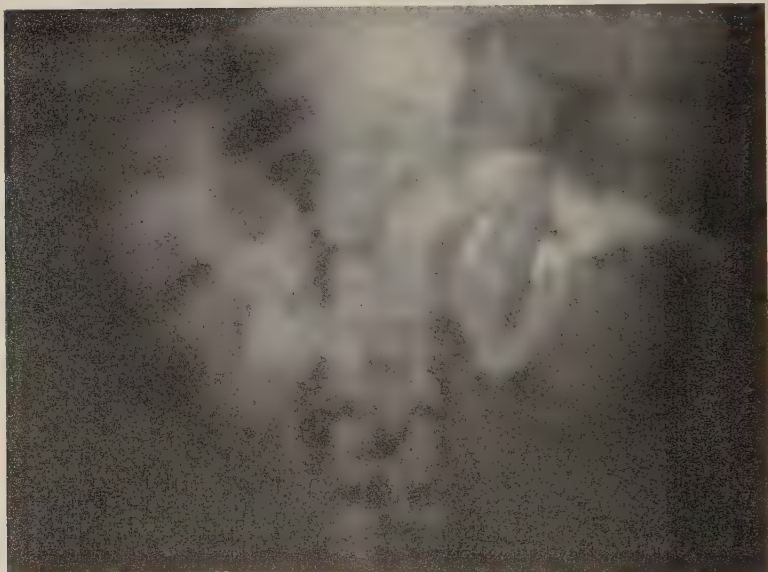


Fig. 4 — Grossly hypertensive pure bilharzial



Fig. 5 — Compensated mixed bilharzial



Fig. 6 — Decompensated mixed bilharzial

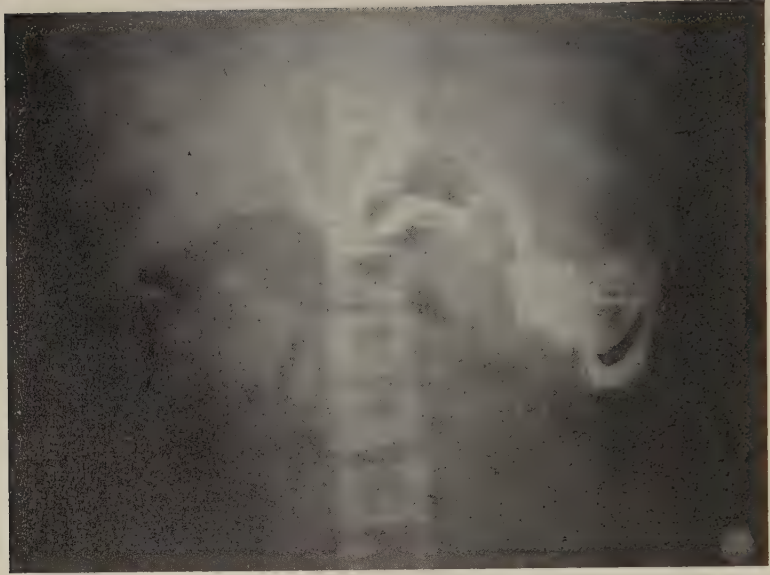


Fig. 7 — Decompensated mixed bilharzial

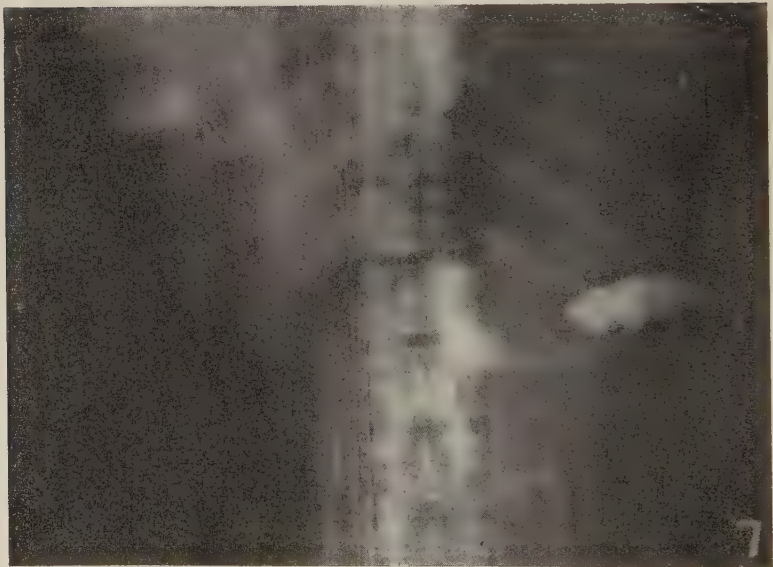


Fig. 8 — Portal vein thrombosis

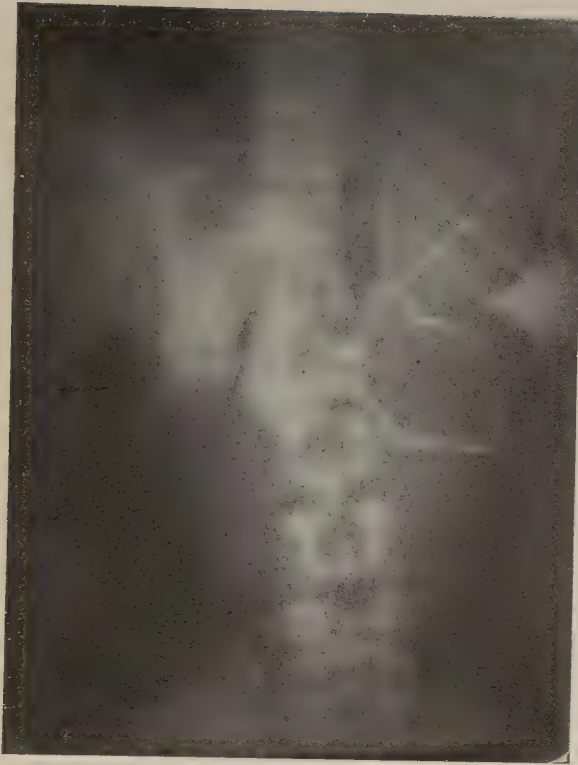


Fig. 9 — Portal and splenic vein thrombosis

THE CIRCUMOVAL TEST IN THE DIAGNOSIS OF INFECTIONS WITH *SCHISTOSOMA MANSONI*

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The antibody precipitin against the eggs of *Schistosoma mansoni* was originally detected in the serum from humans infected with this parasite. Incubation of live eggs in serum resulted in the formation of precipitate around the egg membrane. There are numerous points in connection with this reaction which will be considered.

The antibody appears in the blood of humans around the 40th to the 50th day after exposure to cercariae. This was shown to be the case in a group of 10 children who went bathing and all acquired infection¹. In 2 of them the antibody was present on the 40th day, in 5 of them on the 43rd day and the antibody was detected on the 45th day in the last 3. The reaction was mild, about + at the time of detection, but increased gradually until it became 3 to 4 + about 2 months after exposure to cercariae. The reaction is known to be positive as long as there is an active infection².

At the termination of infection, either as a result of therapy or spontaneous cure, the antibody content decreases or becomes negative.

In a group of individuals who received Fuadin therapy amounting from 100 to 200 cm³, the circumoval test became negative from the 6th to the 8th month after the end of therapy³.

¹ Unpublished report.

² Oliver-González, J., Bauman, P. M., and Benenson, A. S. — «Immunological aspects of infections with *Schistosoma mansoni*», *Amer. Jour. Trop. Med. & Hyg.*, Vol. 4, No. 3, 1955.

³ Oliver-González, J., and Coker, C. M. — «Serological reactions against egg antigens as an aid in the evaluation of therapy in schistosomiasis», *Amer. Jour. Trop. Med. & Hyg.*, Vol. 4, No. 5, 1955.

Recently mice were injected intramuscularly with 10.2 mg of trivalent antimony (Fuadin) per kilogram of body weight, given daily, 5 days a week, for periods up to 280 days. The animals were killed at regular intervals, serum was obtained for the circumoval test, and the tissues examined for the presence of adults and ova. Also, the tissues were fixed for histopathological studies. After the 210th day of treatment the animals were free of their infection with no adult worms. No embryonated or intact eggs were present, but only few empty shells were observed in the tissues. The circumoval test was negative in all of the animals, beginning from the 210th day to the termination of the experiment¹. These findings in mice corroborated previous results in humans in which the circumoval test became negative 6 to 8 months after adequate therapy, and that, therefore, the test may be valuable in determining the therapeutic effect of the drug.

The circumoval test as performed today has some difficulties which cannot be overcome in many laboratories. It requires living eggs and thus the experimental animals to supply them. The test does not give quantitative results, serum titers can not be determined. An antigen from dry eggs material could be used, but it will take large numbers of eggs to make an exceedingly small amount of dry antigen.

Investigations should be continued dealing with preparation of other antigenic extracts proceeding from the egg, adult and cercaria. Also the work by Sherif², in which miracidial antigens give good results in diagnosis and the test becomes negative after therapy, should be extended.

¹ Unpublished report.

² Sherif, A. F. — «A new intradermal antigen for the diagnosis of schistosomiasis», *Ann. Trop. Med. & Parasit.*, June V., 502, 105-112, 1956.

BILHARZIA — SOME PATHOLOGICAL ASPECTS

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The Portuguese Ministry for Overseas Territories has been asked by Dr. Ives Biraud, in his capacity as Director of the Epidemiological Division of W.H.O., to carry out a survey on the geographical distribution of human bilharziasis and its vectors in Portuguese territories in Africa, principally in Guinea, Angola and Mozambique.

It is intended that experts in charge of this survey will collect all those facts which relate to the nature and the extent of bilharzia disease and to the possible danger of the spreading of parasitic infestation as a result of irrigation projects in under-developed countries.

This survey should be especially helpful to local health departments in indicating the extent of the problem with which they have to contend and it should give them some lead as to ways and means of procedure.

A special department was created in our Province for dealing with factors relating to epidemiology and I will not cover this aspect here. My own function lies in the field of pathology and I shall restrict my remarks to that.

In the ten minutes which I am allowed, it would be impossible for me to give you an adequate picture of the pathological pattern of bilharzia disease in Mozambique. I have not time even to describe some of the lesions which I have seen in my daily contact with bilharzia patients over a period of fifteen years.

I will confine myself, for the sake of brevity and of emphasis, to those aspects of the problem which I consider to be the most important.

Let me mention first of all that my work and that of several of my colleagues, in this field, has indicated that almost 100 percent of our native population in Mozambique suffer from bilharzia and very many of them have a lifelong infestation.

This widespread prevalence of schistosoma is evident also in some species of animals — for example, cattle.

It is not surprising then that we find as frequently as we do specific lesions provoked by the worm, its myracidia or its eggs, in almost every organ of the body. Even the skin and the brain are included.

Those research workers who have had the experience of seeing constantly before their eyes the morbidity resulting from the excessive infestation by the bilharzia parasite cannot but be aware of the gravity of the problem involved.

I will cite only such conditions as bilharzia hepatitis, hepatosplenomegaly, serious bladder lesions with maxima hydronephroses and atrophy of the kidneys and carcinoma of the bladder (commonly found), as instance of the damage which the parasite can do. We need have no doubt as to the repercussions of bilharzia infestation in regard to the general metabolism, the hematopoetic and reticulo-endothelial systems and so on.

One continually finds important lesions in almost every organ that is examined. The most serious of these are the lesions of the uro-genital system. They occur nearly always in the southern part of the Province, where the schistosoma haematobium is prevalent, and they are the main cause of the large number of hysterectomies performed on our native women.

We all know that schistosoma haematobium causes very serious lesions of the bladder, ureters and kidneys — for instance, haemorrhagic cystitis, papillomata and so on — and that these lead often to marked atrophy of the kidney tissue, which, in some cases, is represented only by a very thin layer.

Now, in relation to malignant tumours which present one of the most serious medical problems of today, we know, too, that bilharzia plays an important part in the aetiology of carcinoma of the bladder. Time will show what part it plays in the formation of other malignant tumours, for example, the carcinoma of the cervix uteri and of the liver so prevalent amongst the natives of Mozambique — for

bilharzia has serious consequences not only for the uro-genital system. It provokes grave lesions in other vital organs like the liver, where, as the disease progresses, the alterations in the organ can be seen to grow more and more serious.

I am at present, under the auspices of the National Cancer Association of South Africa, conducting a rate survey in Lourenço Marques and I have so far collected specific data covering the past two years.

Examination of this data reveals the startling fact that carcinoma of the liver and liver cirrhosis account for more than one half of the total number of malignant tumours in my area.

Interesting, also, is the fact that the next highest incidence of malignancy appears in the urinary bladder and the cervix uteri.

This occurs not only in my area, but throughout the Province, as is revealed by the data of my ratio study which was published recently by the *British Journal of Cancer*.

An analysis was presented of approximately 2,000 malignant neoplasms diagnosed at autopsy and by histological examination.

Attention is drawn to the high frequency ratio of carcinoma of the liver and bladder in African males. These together account for 51 percent of all tumours in males.

In females, carcinoma of the cervix uteri and of the liver and bladder are responsible for 42 percent of all recorded tumours.

According to research workers in Egypt, bilharzia is the cause of the well-known hepatosplenomegaly and liver cirrhosis which they find; this interests me because, in the cases which I have examined, cirrhosis and primary liver cancer are nearly always associated. Out of some 500 autopsies made every year at my hospital I find approximately 20 percent primary liver cancer and cirrhosis.

From the examination of these cases it seems to me that cirrhosis is a precondition for primary carcinoma of the liver and I wish to draw the attention of other research workers to the correlation between these two conditions.

Finally, in dealing with morbidity in bilharzia disease we must ask ourselves: How is it that we see so varied a pattern? In some cases the lesions are extremely grave and generalized, but in others reactions to the parasite are almost nil.

Now, is this due to the extent of the infestation or to some other contributing factor, e.g., secondary or collateral infections or malnutrition, or does it arise out of the varying effects of different species of the parasite?

This is a matter for our further investigation.

I hope that I have indicated, although briefly, in these few minutes what are to me the most important issues in the pathology of bilharzia disease. My colleagues and I, in Mozambique, are trying to study these problems as intensively as the conditions under which we work will allow us.

I would be very happy if I could convey to you the sense of urgency which we feel about the matter and arouse the interest of the workers in other countries where bilharzia is as great a problem as it is in Mozambique — so that together, and not alone, we may be able to arrive at some solution.

SUMMARY

1. The Health Service of Mozambique has initiated a survey on the geographical distribution of human bilharziasis and its vectors in Portuguese territories in Africa.

2. It is evident from work carried out in the Province that the bilharzia parasite is responsible for a wide range of serious organic lesions not only in the uro-genital system, but in almost every organ of the body, including such vital organs as the liver.

3. I am interested in the work of our Egyptian colleagues who have connected bilharzia with liver cirrhosis because there seems to be indication that, in the natives of Portuguese East Africa (who are almost all afflicted with bilharzia) primary liver cancer may have a cirrhotic basis — and I think this point is worth pursuing.

ESOPHAGEAL VARICES IN MICE INFECTED WITH *SCHISTOSOMA MANSONI*

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A syndrome characterized by hepato-splenomegaly, esophageal varices and ascites occurs in humans infected with *Schistosoma mansoni* (Girges, 1934). Enlargement of the liver and spleen has been reported in laboratory animals experimentally infected with *S. mansoni* (Brumpt and Chevalier, 1931; Koppisch, 1937; Krakower *et al.*, 1943), but esophageal varices have never been described. It has been impossible, therefore, to correlate the experimentally induced disease with the syndrome as it occurs in humans.

The purpose of this communication is to describe the development of esophageal varices in mice infected with *S. mansoni* and to illustrate their relationship to the occurrence of hepatomegaly, splenomegaly and ascites.

MATERIALS AND METHODS

Young adult female Swiss albino mice were individually exposed to 120-130 cercariae of a Puerto Rican strain of *S. mansoni* according

¹ Laboratory of Tropical Diseases.

to the method of Olivier and Stirewalt (1952). The diet consisted of commercial rat pellets fed *ad libitum*. Groups of mice were sacrificed at two-week intervals from the 6th to the 16th week following exposure, and the individual mice were examined for schistosome-induced lesions. In order to preserve esophageal varices for anatomical study a yellow latex medium was injected into a mesenteric vein at a uniform pressure of 200 mm of mercury. The livers were weighed. Spleen indices were determined by multiplying the length (mm) by the width (mm). Worms were recovered by the perfusion method of Yolles *et al.* (1947).

RESULTS

The results of the experiment are summarized in the Table. At 6 weeks no gross consequences of schistosome infection (hepatomegaly,

*Pathological findings in mice infected with Schistosoma mansoni*¹

Weeks after infection	Number of mice examined	Average weight of mice $\pm$ S.E.	Average number of worms per mouse ¹	Average liver weight (g) $\pm$ S.E.	Average spleen index $\pm$ S.E. ²	Number of mice with esophageal varices
0	21	22.9 $\pm$.7	0	1.4 $\pm$.04	186 $\pm$ 7.6	0
6	28	21.5 $\pm$.5	18.6	1.5 $\pm$.05	201 $\pm$ 5.8	0
8	28	19.4 $\pm$.3	32.3	1.9 $\pm$.06	202 $\pm$ 7.0	0
10	28	23.0 $\pm$.3	30.5	2.5 $\pm$.09	245 $\pm$ 7.0	12 (43 %)
12	28	22.8 $\pm$.3	16.3	2.3 $\pm$.08	242 $\pm$ 10.0	8 (29 %)
14	17	24.3 $\pm$.4	21.7	2.4 $\pm$.13	305 $\pm$ 16.2	7 (41 %)
16	28	24.4 $\pm$.3	24.3	2.3 $\pm$.08	254 $\pm$ 11.3	10 (36 %)

¹ Based on worms recovered from 10 animals in each group.

² Spleen index was calculated by multiplying the length (mm) by the width (mm).

splenomegaly, esophageal varices, ascites) were noted. At 8 weeks a significant enlargement of the liver was seen. Splenomegaly was not seen until the 10th week. From this point on hepatomegaly and splenomegaly were found in a significant proportion of the animals. Ascites was present in 7 animals in the 10 and 12 week groups.

Esophageal varices were first seen 10 weeks following infection. 37 of 101 mice (37 percent) examined from the 10th week through

the 16th week had varices. Varices were never seen in uninfected mice or in mice infected 8 weeks or less. The varices differed in both size and distribution, but were all periesophageal. In most cases there was one large tortuous vessel, but in many there were two vessels. These arose from gastric and splenic veins and occasionally from the hepatic venous plexus. They usually terminated in the inferior

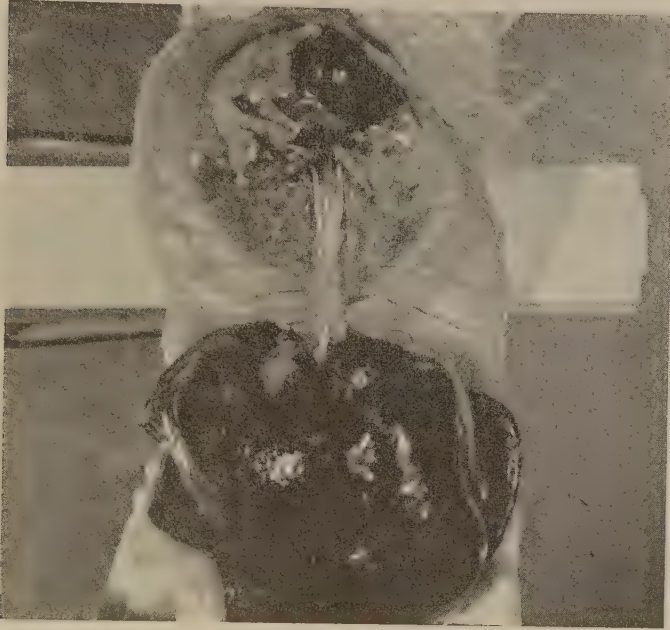


Fig. 1 — An esophageal varix in a mouse exposed to *S. mansoni* 10 weeks previously. The yellow latex dye injected into a mesenteric vein can be noted not only in the varix, but in the blood vessels of the lung and the right ventricle of the heart

vena cava or in the azygos vein. Occasionally they ended in the pulmonary vessels. In one case the anastomosis was so large that latex dye passed from the portal system through the heart, lungs and down the aorta (Fig. 1).

The size of the liver in the mice with esophageal varices was not different from that of comparable mice without varices. The aver-

age spleen size of the mice with varices was always greater than that of the comparable mice without varices, but these differences were not statistically significant.

DISCUSSION

Congestion of the human portal venous system due to intra- or extra-hepatic block is frequently associated with the presence of esophageal varices (Child, 1954). These enlarged blood vessels develop as a collateral pathway due to the impairment of normal hepatic circulation. Their particular significance lies in the fact that bleeding esophageal varices are one of the most frequent causes of mortality in chronic liver disease, including hepato-splenic schistosomiasis *mansoni*.

Esophageal varices are found in a large proportion of patients with hepato-splenic schistosomiasis (Bibawi *et al.*, 1955; Rodriguez *et al.*, 1955). Koppisch (1937), in extensive studies on rats and rabbits infected with *S. mansoni*, did not observe esophageal varices in spite of having examined the esophagus in every case. Krakower *et al.* (1943), in a discussion of portal collateral circulation in guinea pigs infected with *S. mansoni*, note: «There were prominent periesophageal veins [...] but anything even remotely approaching esophageal varices was never seen.»

This is believed to be the first demonstration that esophageal varices caused by schistosomes may occur in an animal other than man. Furthermore, since the varices can be produced experimentally in a laboratory animal, it should be possible to undertake fundamental studies on the pathogenesis and treatment of this important phase of chronic liver disease.

Splenomegaly of the so-called congestive type (Banti's syndrome) is also related to blocked hepatic circulation. It has been shown in this study that hepatomegaly precedes splenomegaly which occurs coincident with the onset of esophageal varices. This points out the relationship of prior liver disease in schistosomiasis to the subsequent development of splenic enlargement and esophageal varices.

SUMMARY

1. Esophageal varices, an integral component of the syndrome of hepato-splenic schistosomiasis *mansoni* in man, have never been described in animals before.
2. Esophageal varices were noted in 37 percent of our mice from 10-16 weeks following infection with *S. mansoni*.
3. The pathology in these *S. mansoni* infected animals may now be related more significantly to that which develops in the human disease.

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FURTHER STUDIES ON THE GROWTH OF *SCHISTOSOMA MANSONI* IN VITRO

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Although prolonged survival of parasitic trematodes under axenic conditions has been reported repeatedly, attempts to obtain growth of these forms in vitro have met with indifferent success. Only in the instance of the attempted cultivation of certain larval strigeids has significant progress been made. It is apparent that successful in vitro cultivation should offer a variety of new opportunities for investigations on the physiology and chemotherapy of this group.

The ease with which immature schistosomes may be recovered free of bacterial contaminants, as well as the inherent importance of these parasites, focused our attention on the suitability of schistosomulae as subjects for growth studies. In 1956, we reported (1) that schistosomulae maintained in vitro with a tissue culture medium supplemented with red blood cells increased significantly in length. More recently, Mao and Lyu (2) and also Robinson (3) have described limited growth of schistosomes under axenic conditions. This communication summarizes further studies in our Department on the growth of immature *Schistosoma mansoni* that have resulted in the development of improved media. Our work has extended to a preliminary examination of the growth-promoting activity of certain of the nutrient components therein. The results will be published in detail elsewhere (4).

METHODS

Schistosomulae were recovered from heparinized white mice 16 to 18 days after the intraperitoneal injection of approximately 200 cercariae of a Puerto Rican strain of *S. mansoni*. Usually the liver and diaphragm was removed en bloc and the immature worms recovered by perfusion of the liver with Hanks' balanced salt solution (5). Under a dissecting microscope, individual schistosomulae were transferred with a Pasteur pipette through fresh changes of perfusion fluid and then to individual rubber-stoppered 15×160 mm culture tubes; these contained 1.5 ml of the medium under investigation and were incubated at 35°C in a vertical position. Determination of the initial size and subsequent growth of individual worms was based on a study of camera lucida outline drawings done at weekly intervals; each length determination represented an average figure calculated from multiple tracings. Only schistosomulae in the initial size range of 0.37-1.33 mm were included in the experiments to be described.

The basic medium consisted of equal parts of horse serum and of Hanks' balanced salt solution together with 100 units of penicillin, 100 µg of streptomycin sulfate and 50 µg of phenol red per milliliter. The nutrient medium was renewed at intervals of 6-8 days and the volume kept constant at 1.5 ml per tube. From 15 to 20 worms, tubed individually, were maintained in each medium under investigation.

RESULTS

Studies were first carried out with media containing equal parts of horse or human serum and the effect of the addition thereto of human red blood cells was investigated. Although added in a 1 percent concentration, the red cells sedimented in the vertical tubes, so that a high concentration of cells was present in the immediate environment of the schistosomulae. It was early noted that the specimens of horse serum available agglutinated human red cells, and that such agglutinated cells were not readily ingested by the schistosomes. Thereafter the agglutinating factor routinely was removed from the horse serum

component by prior absorption at 5°C with a 5 percent volume of fresh human red cells for three consecutive two-day periods. The supplementation of either a 50 percent absorbed horse medium (AHS) or a 50 percent human serum medium (HuS) with 1 percent red cells significantly increased the rate and the amount of growth. For example, at the end of a four-week period of incubation, the average increase in size of worms maintained in AHS was 71 percent and was 335 percent for those in AHS supplemented with red cells.

Attention next was turned to the question of the locus of growth-promoting activity in the red cell. Freshly collected human red cells were lysed by the addition of an equal volume of distilled water and subsequent freezing at -20°C. Acidification then resulted in flocculation of the red cell membranes which were separated by centrifugation. The hemolysate supernatant was saved and subsequently tested in a concentration of 2 percent as a supplement to the AHS medium. The sediment containing the red cell stromata was repeatedly washed, and then added as a supplement in a concentration of 80,000/mm³ of medium; this was equivalent to the concentration achieved by the use of 1 percent packed red cells. The observations in this experiment indicated that most of the growth-promoting material of the red cells was located in the fraction containing the red cell membranes, although the hemolysate supernatant retained some activity. During the first two weeks, the growth rate of worms exposed to intact cells did not differ significantly from that of worms supplemented with red cell stromata; by the end of the fourth week those in AHS-rbc had increased in length 362 percent whereas those in AHS-stromata averaged 289 percent. An additional group of schistosomulae maintained in AHS supplemented with both red cell stromata and red cell hemolysate supernatant showed an average length increase of 423 percent; this, however, on a statistical basis, did not differ significantly from the result obtained with AHS-rbc.

The absorbed horse-serum-red cell medium was further modified in various ways. The optimum growth-promoting modification achieved to date consists of AHS-rbc medium containing added chick embryo extract (CEE) in a final concentration of 10 percent. In two experiments, significantly better growth occurred, with observed size increases of 590 and 483 percent respectively in the groups supple-

mented with CEE and of 426 and 362 percent respectively in the comparable AHS-rbc groups. Several schistosomulae that were approximately 0.4 mm in initial size grew to a length of 7 mm in three weeks in the improved medium containing CEE, and one, initially 1.2 mm long, attained a length of 8.5 mm in the same period. Growth curves derived from representative experiments on schistosomulae cultured in various modifications of the AHS medium are presented in Fig. 1.

In another series of experiments, the activity of certain of the complex defined media now employed for the cultivation of human cells was investigated. When incubated in Mixture # 199 of Morgan, Morton, and Parker (6) schistosomulae did not grow and survived for an average of only five days. The addition of purified globin (0.3 percent), or of crystalline albumin (0.2 percent), to Mixture # 199 prolonged the average survival time to 24 and 14 days respectively. A trial in one experiment of the growth-promoting activity of a medium consisting of equal parts of AHS and of Mixture # 199 resulted in an average increase in worm size of 600 percent in three weeks.

Sexual differentiation of schistosomulae was proportional to the rate of growth and occurred in all media supporting growth. After one month in individual cultures, male and female worms were placed in pairs in culture tubes containing 2-10 ml of medium. Such pairs remained alive in various red blood cell-containing media for several months. They readily copulated and maintained this posture; however, little further increase in length occurred and no ova or sperm were detected at any time in the medium. Examination of stained *toto* mounts showed that whereas the males appeared sexually mature by the end of the fourth week *in vitro*, the females were only partially developed and the vitellaria were rudimentary.

Smyth (7) has emphasized that utilization of the simple criterion of survival of a helminth species under conditions of axenic cultivation may yield fallacious impressions regarding the growth-promoting potential of a particular culture system. In our studies, a variety of media have been shown to vary widely in growth-promoting capacity, but have uniformly permitted survival of schistosomulae for several months under *in vitro* conditions. The best of these, however, still provide suboptimal conditions, for neither full size nor functional

sexual maturity was achieved. Nevertheless, the demonstration that immature *Schistosoma mansoni* under conditions of axenic cultivation

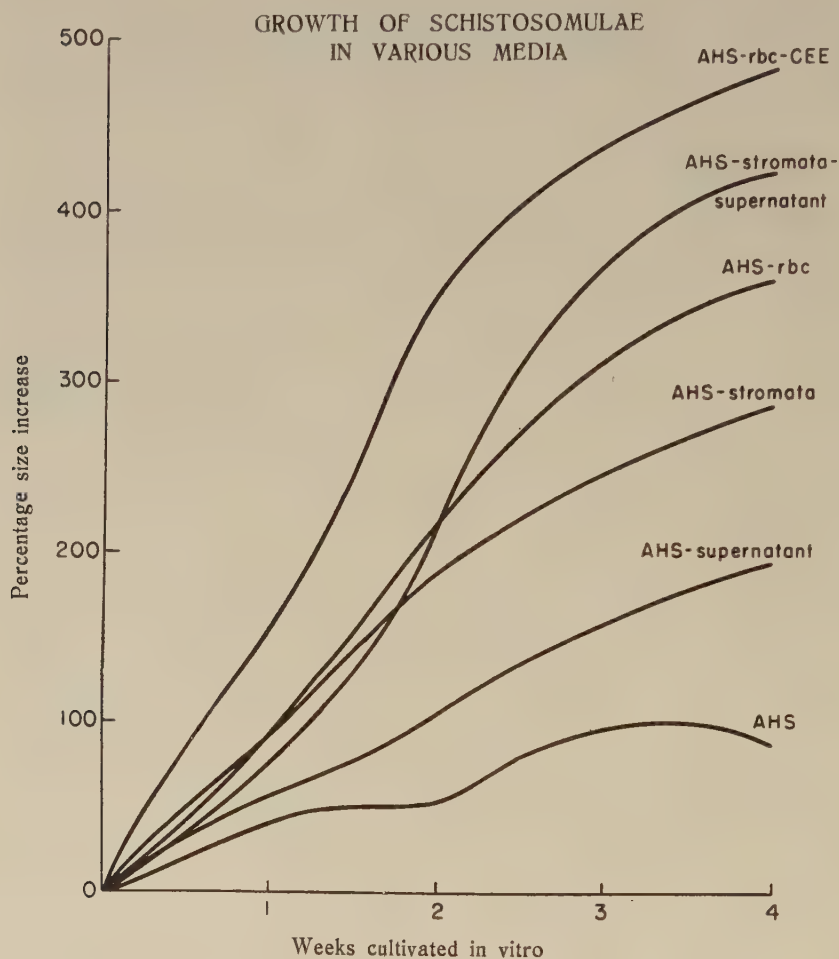


Fig. 1 — Growth curves of schistosomulae maintained in vitro in various modifications of the absorbed horse serum (AHS) red cell medium

will increase in length several hundred percent indicates a degree of progress in the direction of achieving a highly desirable scientific objective.

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STUDIES ON THE BIOLOGY OF *AUSTRALORBIS GLABRATUS*, SCHISTOSOME-BEARING BRASILIAN SNAIL

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The recognition of the need for more knowledge of the schistosome-bearing snails stimulated interest in the procurement of factual and quantitative natural history information, which is essential to an adequate understanding of the epidemiology and control of schistosomiasis. Along with this came increased investigations on the biology of the snail vector. Although there has been a steady interest and a constant accumulation of knowledge, a large proportion dealing with the life history of the *A. glabratus* vector is based on short or casual observations. Therefore, a study on a much greater scale was undertaken and this is the first report of a series which will deal with the biology of the Brazilian *A. glabratus*.

Our snail colonies were kept under conditions which would be regarded inadequate by many investigators, in so far as the rooms in which the snails were housed were under natural illumination and maintained a temperature directly related to normal seasonal fluctuations. Nevertheless, the snails laid eggs and lived sufficiently long for information on the following points: 1) maturity age and maturity size; 2) life cycle; 3) incubation period of eggs; 4) egg-egg mass ratios; 5) viability of eggs in mated and singly snails; 6) hatchability of eggs in relation to seasonal variations of temperature; 7) repro-

¹ Do Instituto Nacional de Endemias Rurais. Núcleo Central de Pesquisas, Rio de Janeiro.

ductive activity in mated and singly snails; 8) fecundity in relation to age and in regard to seasonal variations of temperature; 9) egg and adult progeny per parent snail throughout the reproductive life; 10) population density built up by solitary snails at increasing time intervals; 11) mortality rates in infant, young and old snails; 12) life span; 12) growth curve, and some miscellaneous observations gathered during the three and a half years of experiments which may be of interest and some value in understanding the population dynamics of the Brazilian *A. glabratus*.

MATERIAL AND METHODS

The original pair of wild *A. glabratus* which formed the basis of 15 consecutive laboratory generations in F population was brought from Belo Horizonte (in the State of Minas Gerais) in November 1954. A second colony of 10 consecutive generations in Fx population originated from a pair of wild snails received in June 1956 from the same town but a different locality.

All observations were conducted in three ordinary rooms under normal laboratory illumination and at a temperature influenced by the prevailing weather conditions.

Since a constant supply of biologically balanced water and of oxygenating weeds was necessary, 7 natural ditches measuring 88 cm × 25 cm × 80 cm were dug in the back yard of the laboratory in which *Vallisneria spiralis* and *Elodea canadensis* propagated rapidly. The latter was the weed of choice as a floating oxygenator in the rearing jars without bottom deposit, whereas in the aquaria with bottom deposits *Vallisneria spiralis* quick in growth was also used.

Experiments with paired animals during their entire life were conducted in glass rearing jars measuring either 9 cm in diameter by 11 cm deep or 14 cm by 9 cm. Pairs of snails were placed in the jars when over 10 days old. Each jar contained 600-700 ml of balanced water and several roots of floating weeds for aeration, unless otherwise indicated. Since snails are continuous feeders, pieces of fresh commercial lettuce were introduced daily in excess of what could be consumed. Several drops of a saturated suspension of calcium carbonate were added at weekly intervals unless otherwise indi-

cated. Non-eaten and decaying food was removed, fecal matter was left accumulated at the bottom of the jars. The amount of water lost through evaporation was replaced at weekly intervals. Under the outlined conditions the undisturbed water above the accumulating feces developed a yellow-brown colour but continued clear without any foul smell for over one year. The water was never changed except on the occasions when «bloom» or milky colour developed. Replacements of old rearing jars by fresh ones were rather rare and occurred occasionally during the hot seasons, when, due to solar radiation through the glass windows, an increased population of protozoa and unicellular algae tended to upset the balance of the water. On only one occasion, during the three and a half years of observations, a singly snail became enveloped by a greenish «beard» as reported by Standen (1949).

The snails lived well in the jars, those that died, before they reached sexual maturity, were removed and replaced by live ones of the same size and age from stock. On occasions when one in the paired groups died after the onset of sexual maturity, the remaining one was kept solitary for observations on partially mated specimens.

Snails destined to be observed singly were isolated as soon as they emerged and placed solitary in Petri dishes to ensure complete lack of cohabitation. The dishes were filled to one half capacity with biologically balanced water and a small piece of macerated green lettuce as food was added. At approximately 10 days of age the solitary specimens were removed to rearing jars measuring either 9 cm in diameter by 9.5 cm deep, or 9 cm by 7 cm, and maintained in a manner generally similar to that employed for paired snails except that the water volume was 300-350 ml.

For the experiments concerned with growth in regard to water volume the containers used consisted of three sets of rectangular glass-sided aquaria placed in a separate room. The aquaria measuring 20 cm×39 cm×24 cm or 25 cm×39 cm×27 cm contained 10-12 l of water above a substrate of garden mud, sand and marble chips to a depth of 5-6 cm. Each set consisted of 9 aquaria in which snails, chosen from stock uniform in size, were introduced singly at the age of 30 and 60 days. The aquaria were standardized as to size, substrate, water source, water volume and food allotment. They varied in regard to aerating conditions. One set of 9 aquaria was continuously aerated

from a source of compressed air. In the second set of a similar number of aquaria, oxygenating weeds of *Vallisneria* and *Elodea* were planted. The third set of 9 aquaria represents a combination of the former two; natural oxygenators and continuous artificial aeration were employed. On the occasions when old aquaria were set up afresh, they were left for 7 days for adjustment to laboratory conditions prior to snail introduction.

For growth rate studies, the infant snails were measured microscopically with an ocular micrometer to the nearest 0.01 mm. Larger specimens were measured by hard calipers equipped with a vernier scale to the nearest 0.1 mm.

For the fecundity study egg clutches were removed from each rearing jar and recorded daily including holidays and week-ends until the last snail died.

Egg-egg mass ratios were determined under a dissecting microscope.

Daily records of temperature were kept by the local weather station located approximately 2 km south of the laboratory.

SUMMARY

The experiments described here served to explore several areas simultaneously in two populations of the *A. glabratus* snail, distinct each from the other by the natural habitat of origin. The purposes of the experiments were threefold: 1) the life history of *A. glabratus* in general, 2) the differences between populations of the same species but derived from different original habitats, and 3) the differences between population dynamics of self-fertilized specimens and of those exposed continuously to mating conditions. Whether or not these differences have any real meaning is doubtful, since in nature snails are continuously exposed each to the other. However the knowledge of the population dynamics of solitary snails may have significant implications for operations directed against snails in the field. Of interest was the question of how minimum residual populations would respond to the vacuum created by the control effort, once control measures were suspended or reduced.

The following was found for specimens continuously exposed to mating conditions (a more complete treatment of the subject will appear elsewhere):

1. The preoviposition period averaged 44 days and ranged from 26.9 to 82.7 days in individual generations of snails (Table 1).

2. Size was of greater importance for sexual maturity onset than was age; it averaged 7.7 mm and 8.9 mm in two studied populations (Table 1).

3. Seven generations appeared within over one year, thus indicating an average of 53 days for the life cycle, referring to the period from parents hatching to egg progeny hatching (Table 1).

4. The number of generations per year is a function of two variables: of the preoviposition period and the incubation period. The first is related to the size of snails, which is highly influenced by the environmental conditions, and the second is strongly associated with the seasonal temperature variations.

5. Differences in the incubation period of eggs were coincident with the seasonal variations of temperature. Initial hatchings occurred within a period ranging from 7.3 to 7.9 days, and terminal hatchings within an interval varying from 9.5 to 10.7 days, during the summer season. The maximum average period of first hatchings among eggs laid during the cool season was 15 days and that of last hatchings was 18.6 days (Table 3 and Fig. 1).

6. The number of eggs per egg mass, relatively low on first deposition (cumulative average in paired snails, 8.0-11.2, Table 4), increased gradually through the third month and oscillated thereafter around 19 in one population and around 25 in another population. The egg-egg mass ratio was neither related to age nor to size of parent snails (Table 6 and Figs. 2, 3).

7. Fecundity of snails (egg mass per snail per day) was high during the first months after sexual maturity onset. (Cumulative averages 0.74-0.75 in paired snails.) There was a progressive lowering of reproductive activity with increasing age and a complete breakdown later in life. The maximum length of the reproductive life was approximately 18 months, though sporadic depositions occurred sometimes during the following period (Tables 7, 8 and Fig. 4).

8. The reproductive activity was strongly related to environmental factors conditioned by the calcium availability and by the presence of vegetation (Tables 27, 28). This may be of help in explaining differences in population density in natural foci. The high reproductive activity in the presence of vegetation (Fig. 14) also means that clearance is of importance if reduction of the snail population is being attempted.

9. There was no evidence of a seasonal rhythm in the reproductive activity of laboratory reared snails (Tables 9, 10).

10. The reproductive efficiency of an intermingling population of young and old snails, as referred to the ratio «total number of egg masses-total number of laying specimens», was cyclic in nature. Peaks occurring on alternate years were associated with the prevailing number of highly prolific young generations. Monthly fluctuations were related either to the high incidence of young prolific specimens or to the prevailing number of old snails with a decreased or lost reproductive activity (Tables 11, 12 and Figs. 5, 6).

11. Although the frequency of depositions reflects the reproductive activity of snails, the egg-egg mass ratio is of main value in determining the reproductive potential of snails (Table 13 and Fig. 7).

12. Hatchability of eggs was neither related to the age of parent snails nor to the seasonal variations of temperature (Tables 14, 16 and Figs. 8, 9). The hatchability rates were strongly associated with the incidence of nonviable egg

masses produced by snails. Consequently, it ranged higher, from 62.5 to 91.5 percent, in a population with a low incidence of sterile egg masses, and lower, from 39.0 to 85.2 percent, in another population with a high frequency of nonviable clutches.

13. The incidence of sterile egg masses was neither related to the age of parent snails nor to the environmental conditions (Table 15).

14. While a correlation exists between egg production and potential adult progeny, infant and juvenile mortality seems to be of main importance in evaluating the percentage of eggs laid and hatched that reaches sexual maturity. The adult progeny was markedly higher in one population (assumed 97 vs. 62 adult offspring in the first month ovipositions) than in another despite the similar egg production (385 vs. 372) and because the higher survival rate from egg to adult (25.2 vs. 16.7 percent) (Tables 19, 20).

15. The population density built up by solitary snails was very uneven in ecologically alike artificial foci. The maximum population density was over 100 specimens at the end of 2 months, over 400 snails after 4 months and nearly 700 specimens after 6 months (Table 21). This means that anti-snail measures have to be repeated at short intervals if efficient snail control is being attempted.

16. The reasons underlying the variations in population density occurring between ecologically alike foci (Table 21) are obscure and require further investigations.

17. Snails grew slowly during the first week after hatching and fairly fast through the 40th day. Growth slowed down again following this period and snails seemed to reach a terminal size at the age of 14-17 months (Tables 24, 25 and Figs. 12, 13).

18. The rate of growth and the maximum size attained are variables, strongly associated with the environmental conditions (Tables 26, 28, 29).

19. Since differences in growth and in final size occurred also in parallel breeding of the same snail in the same milieu, the inadvisability of giving too much weight to size as a criterion of age recognition is emphasized.

20. Mortality rates were very high early in life, were moderate in middle life through the eighth month and high again later in life. An estimated rate of 16.7 to 25.2 percent reached sexual maturity (Table 19). From 53.2 to 62.2 percent of adults attained 1 year of age. Four percent lived as long as 2 years and only 1 percent of adults reached 30 months (Table 22).

21. There was no evidence of a seasonal rhythm in mortality rates among the laboratory reared snails (Table 18, Fig. 10).

22. The longest life span of *A. glabratus* was slightly over 32 months. The percentage of hatched that were alive at that time was insignificant, only 0.3. The percentage of hatched that lived nearly 1 year varied from 19.7 to 26.5. Only 2.5 percent of hatched reached the age of approximately 2 years (Table 23, Fig. 11).

23. Parallel breeding of singly snails and of specimens exposed continuously to mating conditions indicates: 1) that the preoviposition period was somewhat longer (Tables 1, 2) and the egg-egg mass ratio on first deposition was lower (by 56 percent) in the former specimens (Table 4); 2) that growth was faster, terminal size (Table 25, Fig. 12) and reproductive activity (Tables 7, 8) greater

in the former than in the latter specimens; and 3) that eggs laid by self-fertilized snails were as viable as those produced by paired specimens (Table 5).

24. Parallel breeding of two populations of the same *A. glabratus* species derived from two different localities, in the same milieu, indicates the following differences in population dynamics: reproductive efficiency (Table 13), hatchability of eggs (Table 16), infant and juvenile survival rates (Table 18), adult progeny (Table 20), and adult survival rates (Table 22) were all higher, while size as referred to shell length was smaller (Tables 25, 26 and Fig. 12) in one population than in the other.

25. Although it is agreed that the knowledge of diverse ecological conditions may be of real help in understanding general and local distribution of snails, and in explaining differences in population dynamics in natural foci, it seems likely that attention must be turned to other features of snails if these problems are to be solved.

TABLE 1 — *Preoviposition period and life cycle in paired A. glabratus*

Date of hatching of successive generations	Generation	Number ¹ of snails tested	Preoviposition period	
			Diameter of snails in millimeters	Age of snails in days
1957				
3-7/3	F ₈	28	10.2 (8.2-12.0)	49.3 (43-63)
3-13/5	F ₉	14	9.9 (8.2-11.8)	82.7 (68-112)
28/7-8/8	F ₁₀	29	7.8 (6.5-10.8)	48.3 (43-58)
1-10/10	F ₁₁	30	9.2 (8.0-11.0)	39.7 (18-50)
17-22/11	F ₁₂	30	8.4 (7.0-9.8)	38.6 (34-44)
1958				
3-8/1	F ₁₃	28	8.7 (7.2-11.0)	30.4 (23-43)
9-12/2	F ₁₄	28	9.1 (8.2-10.2)	32.8 (26-43)
17-24/3	F ₁₅	14	8.3 (6.0-8.8)	42.3 (30-53)
Totals	F ₈ -F ₁₅	201	8.9	42.7
1957				
3-7/3	F _{3x}	30	7.4 (6.5-8.2)	52.2 (44-80)
14/4-7/5	F _{4x}	21	7.7 (6.5-9.0)	71.6 (44-166)
24/6-3/7	F _{5x}	26	7.7 (7.0-9.2)	69.1 (58-77)
17-24/9	F _{6x}	26	7.9 (7.0-8.8)	38.0 (28-58)
5-8/11	F _{7x}	25	7.7 (7.0-8.8)	43.7 (38-54)
21-26/12	F _{8x}	49	7.9 (7.0-9.2)	30.5 (24-44)
1958				
30/1-3/2	F _{9x}	29	7.5 (6.8-8.0)	26.9 (22-34)
5-11/3	F _{10x}	—	—	—
Totals	F _{3x} -F _{9x}	206	7.7	44.6

¹ Uneven numbers are due to death of specimens nearing sexual maturity.

TABLE 2 — *Preoviposition period and life cycle in singly A. glabratus*

Date of hatching of successive generations	Generation	Number of snails tested	Preoviposition period	
			Diameter of snails in millimeters	Age of snails in days
1957				
3-7/3	F ₈	10	10.8 (9.5-13.0)	52.2 (43-64)
6-13/5	F ₉	7	10.4 (9.5-12.2)	70.9 (60-84)
8/8	F ₁₀	10	9.2 (7.5-11.5)	45.7 (40-51)
30/9	F ₁₁	10	11.2 (9.5-12.8)	59.9 (45-67)
13-27/11	F ₁₂	10	10.7 (9.5-12.2)	42.8 (36-52)
1958				
2/1	F ₁₃	9	10.7 (9.8-12.0)	31.6 (25-39)
10/2	F ₁₄	10	10.7 (9.8-11.8)	32.1 (27-43)
17-24/3	F ₁₅	7	9.6 (8.2-11.0)	33.0 (29-35)
Totals	F ₈ -F ₁₅	73	10.4	47.9
1957				
3-7/3	F _{3x}	10	8.2 (7.5-10.5)	52.4 (44-71)
14/4-7/5	F _{4x}	9	7.7 (7.0-8.8)	67.4 (51-89)
24/6-19/7	F _{5x}	9	8.6 (7.2-10.0)	63.2 (42-76)
24/9	F _{6x}	7	8.3 (7.0-9.5)	35.8 (30-54)
5/11	F _{7x}	10	8.4 (7.2-9.5)	41.6 (39-50)
21-26/12	F _{8x}	—	—	—
1958				
27/1	F _{9x}	10	8.6 (8.0-9.2)	30.5 (29-40)
5-11/3	F _{10x}	6	7.6 (6.0-11.0)	57.3 (35-81)
Totals	F _{3x} -F _{10x}	61	8.2	53.0

TABLE 3 — *Effect of temperature variations on the incubation period of A. glabratus eggs*

1957-1958	Average temperature of		Number of clutches examined	Period between laying and hatching in days	
	Max.	Min.		Onset of hatching	End of hatching
May	27.0	15.0	280	13.2 (9-20)	18.3 (16-22)
June	26.6	13.4	392	15.0 (11-22)	16.5 (16-24)
July	25.9	13.9	17	13.5 (13-15)	18.6 (18-22)
August	27.8	13.7	13	13.4 (13-14)	14.7 (13-17)
September	25.7	17.1	146	11.7 (7-14)	13.6 (13-15)
October	30.2	18.5	229	9.3 (7-14)	11.5 (8-14)
November	28.2	18.3	126	10.3 (7-15)	13.5 (9-18)
December	31.5	20.7	154	7.9 (5-12)	10.5 (10-12)
January	33.4	21.0	81	7.6 (6-10)	9.5 (7-12)
February	34.6	20.7	54	7.3 (6-10)	10.7 (7-13)
March	30.3	19.9	55	8.1 (7-11)	11.7 (8-17)
April	28.9	17.9	33	10.5 (6-14)	13.3 (7-19)
May	26.9	16.7	30	14.6 (12-20)	17.4 (12-24)

TABLE 4 — *Average number of eggs per egg-mass in mated and self-fertilized snails at sexual maturity onset*

Generation	Number of 1st ovipositions tested in		Population F		Genera- tion	Number of 1st ovipositions tested in		Population Fx	
			Eggs per egg-mass in					Eggs per egg-mass in	
	Paired	Singly	Paired	Singly		Paired	Singly	Paired	Singly
F ₂	31	14	10.3 (5-22)	5.8 (1-16)	F _{1x}	21	6	15.8 (5-22)	13.6 (8-16)
F ₃	95	11	9.2 (1-19)	4.9 (3-8)	F _{2x}	20	12	9.8 (2-15)	7.9 (5-18)
F ₄	30	—	12.8 (4-22)	—	F _{3x}	40	20	6.2 (4-11)	6.1 (4-8)
F ₅	14	3	16.3 (10-21)	4.3 (2-5)	F _{4x}	14	6	8.3 (5-15)	7.0 (5-8)
F ₆	28	4	8.2 (1-17)	5.7 (4-8)	F _{5x}	19	9	5.5 (1-12)	5.8 (2-13)
F ₇	16	5	13.3 (4-25)	7.2 (3-12)	F _{6x}	15	5	12.4 (8-15)	8-0 (3-20)
F ₈	34	15	16.1 (8-21)	7.3 (2-13)	F _{7x}	8	13	11.5 (9-16)	11.2 (7-17)
F ₉	9	7	8.1 (5-12)	5.1 (5-6)	F _{8x}	40	—	4.9 (1-13)	—
F ₁₀	29	10	9.5 (2-19)	6.2 (3-7)	F _{9x}	26	28	5.8 (3-8)	5.2 (2-14)
F ₁₁	16	6	14.1 (3-25)	4.0 (4-6)	—	—	—	—	—
F ₁₂	20	—	10.7 (6-19)	—	—	—	—	—	—
F ₁₃	25	11	10.7 (2-17)	5.8 (2-10)	—	—	—	—	—
F ₁₄	25	13	13.0 (5-20)	5.4 (2-8)	—	—	—	—	—
F ₁₅	12	13	7.0 (2-11)	6.5 (3-10)	—	—	—	—	—
Totals. . .	384	112	11.2	5.8	—	203	99	8.0	7.2

Cumulative average of eggs on first deposition in two populations: paired — 10.1

Cumulative average of eggs on first deposition in two populations: singly — 6.5

TABLE 5 — *Comparative data on the average number of eggs per egg-mass laid by paired and singly snails*

Month after 1st oviposition		2nd		3rd		4th		5th	
Generation	Mode of maintenance	Number of egg-masses examined	Mean number of eggs per egg-mass	Number of egg-masses examined	Mean number of eggs per egg-mass	Number of egg-masses examined	Mean number of eggs per egg-mass	Number of egg-masses examined	Mean number of eggs per egg-mass
F _{7x} . . .	Paired . . .	75	24.1	70	33.4	43	32.1	29	28.7
	Singly . . .	58	24.5	49	20.4	37	22.2	28	29.4
F _{9x} . . .	Paired . . .	73	20.1	42	21.3	—	—	—	—
	Singly . . .	29	17.0	30	29.2	—	—	—	—
F ₁₂ . . .	Paired . . .	128	22.5	122	26.4	54	25.7	63	21.5
	Singly . . .	57	11.7	53	11.7	29	11.3	39	12.5
F ₁₄ . . .	Paired . . .	95	19.7	29	22.0	—	—	—	—
	Singly . . .	65	11.2	57	18.0	—	—	—	—

TABLE 6 — *Effect of age and size of A. glabratus on the number of eggs in egg-masses*

Month after first oviposition	Population F				Population Fx			
	Number of snails tested	Mean length of shell in millimeters	Total number of egg-masses examined	Number of eggs per egg-mass, mean	Number of snails tested	Mean length of shell in millimeters	Total number of egg-masses examined	Number of eggs per egg-mass, mean
* 0	575	9.4	433	9.9	297	8.1	248	8.3
1	574	11.9	3,544	14.7	264	9.5	1,745	15.1
2	379	13.8	1,604	18.2	207	10.8	1,158	24.5
3	339	15.0	1,245	20.4	126	11.9	598	23.1
4	312	15.6	1,088	19.0	111	12.4	344	26.6
5	224	16.1	715	18.4	97	13.0	314	29.1
6	199	16.4	542	18.2	76	13.8	274	30.1
7	147	17.2	412	17.5	67	14.2	226	28.5
8	113	17.2	364	18.2	49	14.8	198	23.7
9	83	18.7	341	21.6	22	14.8	46	27.7
10	77	18.5	257	22.3	26	15.2	61	26.8
11	52	19.1	121	22.9	20	15.3	48	18.1
12	41	19.2	107	20.2	11	15.5	18	21.5
13	30	19.3	81	20.6	—	—	—	—
14	25	18.9	73	24.3	—	—	—	—
15	8	19.2	18	20.0	—	—	—	—
18-20	4	19.2	9	18.2	—	—	—	—

* First oviposition.

TABLE 7 — *Comparative fecundity of continuously mated, partially mated and singly A. glabratus (population F)*

Month after first oviposition	Mode of maintenance								
	Paired			Partially paired			Singly		
	Number of snails tested	Total number of egg-masses	Mean egg-mass per snail per day	Number of snails tested	Total number of egg-masses	Mean egg-mass per snail per day	Number of snails tested	Total number of egg-masses	Mean egg-mass per snail per day
1	424	9,565	0.75	16	366	0.76	88	3,439	1.30
2	342	7,678	0.75	21	717	1.14	75	2,968	1.32
3	300	6,062	0.67	31	1,246	1.34	63	2,051	1.08
4	282	5,223	0.62	30	913	1.01	54	1,636	1.01
5	228	3,765	0.55	30	751	0.83	45	1,097	0.81
6	202	3,127	0.52	32	616	0.64	36	713	0.66
7	178	2,170	0.41	20	405	0.67	29	385	0.44
8	136	1,620	0.39	10	160	0.53	23	186	0.27
9	126	1,639	0.43	8	199	0.83	21	78	0.12
10	116	1,398	0.40	7	196	0.93	19	29	0.05
11	92	989	0.36	4	123	1.02	15	40	0.09
12	70	761	0.36	6	120	0.67	12	98	0.27
15	28	209	0.25	5	62	0.41	4	32	0.27
18	10	53	0.18	—	—	—	2	0	0

TABLE 8 — *Comparative fecundity of mated and singly A. glabratus (population Fx)*

Month after first oviposition	Mode of maintenance					
	Paired			Singly		
	Number of snails tested	Total number of egg-masses	Mean egg-mass per snail per day	Number of snails tested	Total number of egg-masses	Mean egg-mass per snail per day
1	234	5,179	0.74	55	1,486	0.90
2	204	3,985	0.65	42	1,111	0.88
3	138	2,454	0.59	40	985	0.82
4	124	1,840	0.49	31	679	0.73
5	94	1,436	0.51	27	648	0.80
6	86	1,262	0.49	25	544	0.73
7	68	906	0.44	20	441	0.73
8	55	651	0.39	18	399	0.74
9	50	637	0.42	15	251	0.56
10	44	461	0.35	15	144	0.32
11	24	259	0.36	8	92	0.38
12	18	124	0.23	8	95	0.40
13	8	35	0.15	6	70	0.39
14	—	—	—	5	20	0.13

TABLE 9 — Average daily egg-mass deposition in successive laboratory reared generations of *A. glabratus*, recorded monthly during a period of three years (F population)

Month	1955								1956								1957																		
	Generation								Generation								Generation																		
	F ₁		F ₂		F ₃		F ₄		F ₃		F ₃		F ₄		F ₅		F ₆		F ₄		F ₅		F ₆		F ₇		F ₈		F ₉		F ₁₀		F ₁₁		
	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	Number of snails tested	Mean egg-mass per snail per day	
January . . .	32	0.82	—	—	—	—	—	—	14	0.27	20	0.39	30	0.58	—	—	—	—	6	0.37	18	0.84	32	0.65	—	—	—	—	—	—	—	—	—	—	—
February . .	30	0.73	—	—	—	—	—	—	8	0.22	18	0.33	30	0.61	—	—	—	—	6	0.21	18	0.81	32	0.72	20	0.80	—	—	—	—	—	—	—	—	
March . . .	28	0.57	32	0.52	—	—	—	—	6	0.20	16	0.24	28	0.69	—	—	—	—	6	0.11	18	0.71	32	0.68	18	0.64	—	—	—	—	—	—	—	—	
April	28	0.54	30	0.70	—	—	—	—	6	0.13	12	0.08	24	0.61	—	—	—	—	6	0.09	14	0.57	30	0.62	20	0.54	—	—	—	—	—	—	—	—	
May	26	0.47	30	0.71	—	—	—	—	6	0.11	10	0.16	22	0.34	14	0.59	—	—	4	0.01	12	0.60	28	0.59	18	0.55	30	1.18	—	—	—	—	—	—	
June	24	0.26	30	0.63	84	0.52	—	—	6	0.05	8	0.08	20	0.17	14	0.35	—	—	—	—	12	0.46	26	0.53	12	0.60	26	1.09	—	—	—	—	—	—	
July	24	0.13	30	0.43	68	0.53	—	—	6	0.06	8	0.12	18	0.11	14	0.33	—	—	—	—	12	0.44	22	0.48	8	0.49	26	0.94	—	—	—	—	—	—	
August . . .	20	0.15	30	0.46	68	0.58	—	—	4	0.01	4	0.11	12	0.05	14	0.26	—	—	—	—	10	0.33	22	0.49	6	0.40	24	0.72	20	0.71	—	—	—	—	
September . .	20	0.08	30	0.38	64	0.48	—	—	4	0.01	4	0.03	12	0.02	12	0.27	—	—	—	—	4	0.23	22	0.48	6	0.60	16	0.82	12	0.97	—	—	—	—	
October . . .	14	0.03	28	0.39	64	0.49	—	—	4	0.10	2	0.02	12	0.05	10	0.27	—	—	—	—	2	0.83	22	0.55	4	0.89	16	1.23	8	1.05	26	1.23	—	—	
November . .	12	0.02	24	0.38	56	0.46	—	—	1	0	—	—	8	0.08	10	0.38	—	—	—	—	—	—	20	0.54	4	0.59	16	0.98	8	0.94	26	1.06	30	0.79	
December . .	8	0.01	22	0.40	40	0.37	44	0.52	1	0	—	—	8	0.08	12	0.56	38	0.35	—	—	—	—	14	0.47	2	1.10	10	0.95	8	0.81	16	0.91	30	0.91	

TABLE 11 — Summary of data showing fecundity rates in a pooled population of young and old *A. glabratus*, determined monthly during a period of three years (F population)

Month	Range of mean temperature 1955, 1956, 1957		1955		1956		1957		Cumulative total of snails tested	Cumulative mean egg-mass per snail per day
	Max.	Min.	Total number of snails tested	Mean egg-mass per snail per day	Total number of snails tested	Mean egg-mass per snail per day	Total number of snails tested	Mean egg-mass per snail per day		
January	30.7-35.6	20.2-20.3	32	0.82	64	0.45	56	0.63	152	0.61
February	31.7-33.4	19.4-20.9	30	0.73	56	0.46	76	0.72	162	0.63
March	31.4-32.1	19.6-20.2	60	0.54	50	0.49	74	0.63	185	0.56
April	28.5-29.2	18.0-18.7	58	0.63	42	0.39	70	0.54	170	0.53
May	25.1-27.0	15.0-19.3	56	0.60	52	0.35	92	0.75	200	0.60
June	24.5-26.6	13.4-14.0	138	0.50	48	0.19	76	0.72	262	0.50
July	25.3-27.1	12.6-14.2	122	0.43	46	0.17	68	0.65	236	0.44
August	24.6-27.8	12.3-13.7	118	0.43	34	0.14	82	0.58	234	0.46
September	25.7-28.1	14.4-17.1	114	0.38	32	0.12	60	0.66	206	0.42
October	25.0-30.2	16.0-18.5	106	0.40	28	0.12	78	0.99	212	0.58
November	26.8-28.2	16.6-18.3	92	0.39	19	0.23	104	0.84	215	0.59
December	28.7-31.5	18.0-20.7	114	0.41	59	0.35	80	0.83	253	0.53
Cumulative totals			1,040	0.48	530	0.32	916	0.73		

TABLE 12 — *Summary of data showing fecundity rates in a pooled population (F_x) of young and old A. glabratus, determined monthly through 1957*

Month	Total number of snails tested	Total number of egg-masses	Mean egg-mass per snail per day
January	30	264	0.29
February	62	1,070	0.57
March.	54	1,023	0.63
April	46	632	0.46
May	70	1,233	0.59
June	66	1,016	0.51
July.	84	947	0.38
August	76	759	0.33
September	94	1,344	0.48
October	92	1,551	0.56
November	106	1,684	0.53
December	108	2,198	0.68
Cumulative totals . . .	888	13,721	0.51

TABLE 13 — *Reproductive potential of two populations of A. glabratus*

Month after 1st oviposition	1st		2nd		3rd		4th		5th		6th		7th	
Population	Egg-mass per snail per day	Eggs per snail per day	Egg-mass per snail per day	Eggs per snail per day	Egg-mass per snail per day	Eggs per snail per day	Egg-mass per snail per day	Eggs per snail per day	Egg-mass per snail per day	Eggs per snail per day	Egg-mass per snail per day	Eggs per snail per day	Egg-mass per snail per day	Eggs per snail per day
F	0.84	12.4	0.86	15.7	0.79	16.1	0.74	13.4	0.62	11.4	0.55	10.1	0.45	7.9
Fx	0.77	12.8	0.69	17.0	0.64	18.1	0.55	14.5	0.57	17.3	0.54	16.3	0.43	12.3

Month after 1st oviposition	8th		9th		10th		11th		12th		15th		18th	
Population	Egg-mass per snail per day	Eggs per snail per day	Egg-mass per snail per day	Eggs per snail per day	Egg-mass per snail per day	Eggs per snail per day	Egg-mass per snail per day	Eggs per snail per day	Egg-mass per snail per day	Eggs per snail per day	Egg-mass per snail per day	Eggs per snail per day	Egg-mass per snail per day	Eggs per snail per day
F	0.39	7.0	0.44	9.5	0.38	8.5	0.35	7.9	0.37	7.5	0.27	5.5	0.24	4.3
Fx	0.47	11.2	0.45	12.6	0.34	9.1	0.38	6.6	0.27	5.7	—	—	—	—

TABLE 14—*Effect of increasing age of parent-snails on the viability of eggs*

Month after 1st oviposition	Population F			Population Fx		
	Number of egg-masses examined	Total number of eggs	Percent of hatched	Number of egg-masses examined	Total number of eggs	Percent of hatched
1	1,167	16,413	54.7	787	8,892	70.6
2	975	20,165	63.4	951	22,805	81.5
3	671	16,208	57.1	554	14,434	83.6
4	701	14,003	60.5	468	13,222	86.0
5	353	6,889	49.5	297	8,818	77.2
6	318	6,899	59.8	314	9,650	75.9
7	165	3,638	65.6	223	5,548	75.5
8	150	3,053	59.1	127	4,127	66.1
10-11 . . .	63	1,058	85.9	30	513	96.3

TABLE 15—*Incidence of nonviable eggs determined at monthly intervals after first oviposition*

Month	1st		2nd		3rd		4th		5th	
Generation	Total number of eggs examined	Percentage of nonviable eggs	Total number of eggs examined	Percentage of nonviable eggs	Total number of eggs examined	Percentage of nonviable eggs	Total number of eggs examined	Percentage of nonviable eggs	Total number of eggs examined	Percentage of nonviable eggs
F _{6x}	764	2.9	1,468	6.5	1,657	5.8	731	2.3	645	10.4
F _{7x}	603	1.5	3,263	1.9	3,376	6.9	2,335	0.8	1,826	6.8
F _{8x}	887	6.8	5,250	5.9	1,432	1.7	1,147	0.3	—	—
F ₁₂	642	24.4	3,564	9.5	4,124	35.5	1,816	33.1	2,226	40.8
F ₁₃	1,303	15.6	3,067	23.7	2,272	25.4	1,892	7.2	—	—

TABLE 16 — *Fertility of A. glabratus egg-masses determined after the 17th of each¹ month from February 1957 until March 1958*

Period of observations 1957-1958	Population F					Population Fx		
	Average temperature		Number of egg- masses examined	Total number of eggs in egg-masses	Percent of hatched	Number of egg- masses examined	Total number of eggs in egg-masses	Percent of hatched
	Max.	Min.						
February	31.7	20.9	264	6,402	85.2	196	5,349	81.6
March	31.4	20.2	53	1,288	75.8	47	1,580	85.3
April	28.6	18.7	349	7,044	61.3	329	6,979	71.6
May	27.0	15.0	425	8,365	68.9	342	8,261	71.3
June	26.6	13.4	413	10,234	70.7	356	9,168	77.2
September.	25.7	17.1	544	7,589	67.6	484	10,462	91.2
October.	30.2	18.5	414	7,443	46.8	369	10,155	80.6
November.	28.2	18.3	405	7,974	56.7	265	6,675	83.9
December	31.5	20.7	272	5,017	63.3	280	5,934	89.0
January	33.4	21.0	352	7,531	41.1	376	7,321	88.5
February	34.6	20.7	656	11,842	39.0	416	11,023	62.5
March	30.3	19.9	240	5,252	49.7	177	4,328	87.8

¹ Lack of data in July and August is due to the use of egg-masses in experiments on total weight and shell weight relationship.

TABLE 17 — *Fertility of egg-masses laid by mated and self-fertilized A. glabratus*

Generation	Mode of maintenance	Number of egg-masses examined	Total number of eggs	Hatched eggs		Non-fertile depositions				Percentage of non-hatched eggs in fertile clutches
				Number	Percentage	Egg-masses		Eggs		
						Number	Percentage	Number	Percentage	
F _{5x}	Paired . .	307	9,138	7,326	80.2	39	12.7	1,078	11.8	8.0
	Singly . .	80	1,325	1,174	88.6	9	11.9	71	5.3	6.1
F _{6x}	Paired . .	207	4,945	3,894	78.7	26	12.5	553	11.2	10.1
	Singly . .	25	345	261	75.6	4	16.0	65	18.8	5.6
F _{7x}	Paired . .	258	6,860	5,422	79.0	42	16.2	790	11.5	9.5
	Singly . .	142	4,385	3,596	82.0	25	17.6	501	11.4	6.6
F _{9x}	Paired . .	199	3,356	2,896	86.3	36	18.1	276	8.2	5.5
	Singly . .	108	1,736	1,443	83.1	27	25.0	173	9.9	7.0

TABLE 18—*Percentage of living snails counted at the age of 3-15 days and 18-30 days*

Period of observations, ¹ 1957-1958	Average temperature		Population F						Population Fx					
			Total number of eggs tested	Number of hatched snails	Percent of living snails on day				Total number of eggs tested	Number of hatched snails	Percent of living snails on day			
	3-15				18-30		3-15				18-30			
	Max.	Min.			Upon	Hatched	Upon	Hatched			Upon	Hatched	Upon	Hatched
	Eggs	Upon	Hatched	Eggs	Upon	Hatched	Eggs	Upon	Hatched	Eggs	Upon	Hatched	Eggs	Upon
February . . .	31.7	20.9	6,402	5,454	46.1	54.1	26.7	31.4	5,349	43.0	52.7	27.2	33.4	
March	31.4	20.2	1,288	977	28.4	31.1	—	—	1,580	29.6	34.7	—	—	
April	28.6	18.7	7,044	4,319	30.4	49.6	12.5	18.2	6,979	44.4	62.0	26.8	37.4	
May	27.0	15.0	8,365	5,763	35.1	51.0	18.3	26.5	8,261	48.6	68.2	31.7	44.5	
June	26.6	13.4	10,234	7,238	42.6	60.2	23.9	33.9	8,474 *	53.0	67.3	42.2	53.5	
September. . .	25.7	17.1	7,589	4,134	33.7	61.9	27.1	49.8	10,462	67.6	73.9	51.3	56.1	
October. . . .	30.2	18.5	7,992 *	3,866	25.1	52.0	11.5	23.8	10,155	56.1	74.5	45.3	60.1	
November. . .	28.2	18.3	7,425 *	4,141	26.6	47.6	19.7	35.3	6,675	50.4	60.1	29.9	35.6	
December. . .	31.5	20.7	5,017	3,178	32.0	50.6	25.9	40.7	5,984	63.0	70.8	57.4	64.4	
January. . . .	33.4	21.0	7,531	3,094	18.3	44.5	14.7	35.7	7,321	61.8	69.8	48.8	55.2	
February . . .	34.6	20.7	12,062 *	4,634	12.7	33.1	10.0	25.9	11,023	31.3	50.1	20.6	32.9	
March	30.3	19.9	5,252	2,609	27.4	55.2	21.7	43.7	4,328	70.5	80.4	30.2	34.4	

¹ Referred to egg collections.
See text for details.

TABLE 19 — *The proportion of eggs and hatched reaching sexual maturity*

Generation	Number of eggs laid ¹	Number of hatched ¹	Living snails between 18-30 day after hatching			Living snails at sexual maturity onset			
			Number	In percent of eggs	In percent of hatched	Number	In percent of eggs	In percent of hatched	In percent of 18-30 days old
F ₃	981	487	213	21.7	43.7	138	14.1	28.3	64.8
F ₄	262	142	71	27.1	49.8	53	20.2	37.3	74.6
F ₅	157	78	34	21.7	43.7	30	19.1	38.5	88.2
F ₇	330	213	63	19.1	29.6	55	16.7	25.8	87.3
F ₈	188	162	48	25.5	29.6	40	21.3	24.7	83.3
F ₉	288	198	36	12.5	18.2	33	11.5	16.7	91.7
F ₁₀	167	118	40	23.9	33.9	40	23.9	33.9	100.0
F ₁₁	279	118	41	14.7	34.7	40	14.3	33.9	97.5
F ₁₂	160	100	40	25.0	40.1	40	25.0	40.0	100.0
Totals . . .	2,803	1,616	586	20.9	36.3	469	16.7	29.0	80.0
F _{2x}	131	127	70	53.4	55.1	49	37.4	38.6	70.0
F _{3x}	127	105	40	31.5	38.2	40	31.5	38.1	100.0
F _{4x}	317	107	39	12.3	36.4	36	11.3	33.6	92.3
F _{5x}	97	77	41	42.2	53.5	37	38.1	48.0	90.2
F _{6x}	76	68	40	52.7	59.0	37	48.7	54.4	92.5
F _{7x}	192	139	54	28.1	38.8	38	19.8	27.3	70.4
Totals . . .	940	623	284	30.2	45.6	237	25.2	38.0	83.4

¹ Estimated numbers.

TABLE 20 — Potential egg and adult progeny per snail in two populations of *A. glabratus*, determined at increasing time intervals after first oviposition

Months after 1st oviposition		1	2	3	4	5	6	7	8	9	10	11	12	13
Population F	Total number of													
	Egg-masses ¹	25	51	75	96	115	131	145	156	170	181	191	202	254
	Eggs ¹	372	843	1,327	1,730	2,072	2,372	2,610	2,821	3,106	3,360	3,599	3,823	4,877
Population Fx	Total number of													
	Egg-masses	23	44	63	79	97	113	126	140	154	164	175	183	—
	Eggs	385	894	1,436	1,872	2,393	2,883	3,254	3,590	3,970	4,243	4,443	4,613	—
Population Fx	Adult offspring . . .	97	225	362	472	603	727	820	905	1,000	1,069	1,120	1,163	—

¹ Experimental data.

² Theoretical data.

TABLE 21 — *The population density built up by singly snails maintained under varying laboratory conditions*

Snails counted on day after introduction	Oxygenating weeds + —	Artificial aeration + —	Total number of aquaria tested	Number of aquaria with new populations	Number of recovered snails	
					Living	Dead
60	+	+	8	7	27 (5-81)	1 (0-4)
	+	—	7	5	36 (6-119)	0
	—	+	7	4	49 (5-80)	1 (0-5)
120	+	+	8	5	196 (33-428)	3 (0-12)
	+	—	23	10	21 (6-70)	0 (0-2)
	—	+	8	3	24 (11-34)	4 (0-10)
	—	—	12	6	7 (2-15)	0 (0-2)
180	+	+	9	6	25 (6-73)	0 (0-1)
	+	—	18	12	159 (23-631)	2 (0-9)
	—	+	9	9	75 (16-150)	5 (0-19)
	—	—	7	7	28 (16-52)	4 (0-11)

TABLE 22 — *Number of snails living at increasing time intervals after sexual maturity onset in successive laboratory reared generations of two A. glabratus populations*

Generation	Number of snails examined	Number of living snails at the end of each period										
		(542)* 3	(542) 6	(474) 9	(434) 12	(415) 15	(398) 18	(343) 21	(299) 24	(299) 27	(269) 30	(269) 33
F ₁	36	33	30	28	19	8	6	4	0	0	0	0
F ₂	52	49	48	42	31	23	14	9	6	4	3	0
F ₃	130	120	106	91	70	35	15	5	2	2	0	0
F ₄	51	43	35	22	19	16	10	5	2	0	0	0
F ₅	30	28	24	23	21	20	11	6	2	1	—	—
F ₆	44	37	34	31	27	24	18	16	—	—	—	—
F ₇	55	47	38	30	22	19	14	12	—	—	—	—
F ₈	17	16	16	13	10	9	—	—	—	—	—	—
F ₉	19	17	16	15	12	—	—	—	—	—	—	—
F ₁₁	40	38	29	28	—	—	—	—	—	—	—	—
F ₁₂	35	33	27	—	—	—	—	—	—	—	—	—
F ₁₃	33	29	23	—	—	—	—	—	—	—	—	—
Total of survivors . .	Number	490	426	323	231	154	88	57	12	7	3	0
	Percent	90.4	78.6	68.1	53.2	37.1	22.1	16.6	4.0	2.3	1.0	0
		(316)	(316)	(201)	(201)	(128)						
F _{1x}	39	36	32	31	26	23	—	—	—	—	—	—
F _{2x}	49	36	33	29	27	21	—	—	—	—	—	—
F _{3x}	40	39	36	33	28	24	—	—	—	—	—	—
F _{4x}	36	32	23	23	23	—	—	—	—	—	—	—
F _{5x}	37	27	24	24	21	—	—	—	—	—	—	—
F _{6x}	37	22	14	—	—	—	—	—	—	—	—	—
F _{7x}	38	35	35	—	—	—	—	—	—	—	—	—
F _{8x}	40	37	33	—	—	—	—	—	—	—	—	—
Total of survivors . .	Number	264	230	140	125	68	—	—	—	—	—	—
	Percent	83.5	72.8	69.6	62.2	53.1	—	—	—	—	—	—

* Figures in parentheses indicate cumulative number of snails at the beginning of each period.

TABLE 23 — *Life span of A. glabratus expressed in numbers of survivors in an actual group of matures and in percent of survivors in theoretical groups of eggs and of hatched*

Month after egg deposition	Survivors at the end of each period			Survivors at the end of each period		
	Number	Percent upon		Number	Percent upon	
		Eggs	Hatched		Eggs	Hatched
0	1,611	100	—	798	100	—
0.5	928	57.6	100	529	66.3	100
2	269	16.7	29.0	201	25.2	38.0
5	245	15.2	26.4	170	21.3	32.1
8	219	13.6	23.6	148	18.6	28.0
11	183	11.3	19.7	140	17.5	26.5
14	139	8.6	13.9	125	15.7	23.6
17	82	5.1	8.8	—	—	—
20	45	2.8	4.8	—	—	—
23	23	1.4	2.5	—	—	—
26	10	0.6	1.1	—	—	—
29	6	0.4	0.6	—	—	—
32	3	0.2	0.3	—	—	—
35	0	0	0	—	—	—

TABLE 24—*Comparative mean shell measurements of infant and young A. glabratus maintained in different conditions*

Age in days	Total number measured	Diameter in millimeters
0-1	100	0.59 (0.51-0.69)
3-4	100	0.77 (0.64-1.06)
6-7	100	0.81 (0.70-1.08)
10	100	1.03 (0.83-1.3)
10*	90	1.28 (0.64-1.8)
20	100	1.8 (1.2-2.4)
20*	90	3.1 (1.5-5.2)
30	98	2.4 (1.6-3.5)
30*	83	5.1 (2.5-8.0)
40	48	3.7 (1.9-5.5)
40*	80	7.3 (4.0-10.2)

* Maintained singly in 300-350 ml of water.

TABLE 25 — *Monthly shell measurements of laboratory reared A. glabratus*

Age of snails in months	Population F				Population Fx			
	Mode of maintenance				Mode of maintenance			
	Paired		Singly		Paired		Singly	
	Number of snails tested	Mean diameter in millimeters	Number of snails tested	Mean diameter in millimeters	Number of snails tested	Mean diameter in millimeters	Number of snails tested	Mean diameter in millimeters
2	470	9.1 (5.0-16.8)	96	10.9 (7.5-18.5)	234	7.9 (6.5-11.2)	63	8.5 (7.0-14.2)
3	422	11.3 (5.5-17.5)	88	13.9 (9.5-20.0)	236	9.5 (6.5-11.5)	54	10.1 (7.5-15.0)
4	358	13.2 (6.2-20.5)	69	15.4 (11.8-21.0)	216	10.7 (7.0-15.5)	53	11.0 (8.2-14.8)
5	334	14.8 (6.8-22.0)	61	16.3 (11.8-21.5)	150	11.5 (7.0-15.5)	43	12.1 (9.0-15.5)
6	276	15.7 (9.2-22.8)	45	17.2 (13.5-21.5)	122	12.2 (7.5-16.5)	31	12.5 (9.0-15.8)
7	236	16.1 (9.5-23.0)	41	17.7 (14.2-22.8)	98	13.1 (9.5-16.8)	27	13.3 (10.8-16.5)
8	210	16.4 (10.0-23.0)	32	17.7 (14.8-21.2)	86	13.6 (10.2-17.2)	25	14.1 (11.2-16.5)
9	186	17.0 (11.0-23.0)	27	18.4 (14.8-22.0)	68	14.0 (10.2-17.5)	20	14.8 (12.5-18.0)
10	140	17.6 (13.0-22.5)	22	18.8 (14.8-22.0)	56	14.6 (10.5-17.5)	18	15.2 (13.2-16.8)
11	124	18.3 (13.5-24.0)	19	19.3 (14.8-21.5)	50	14.9 (11.2-17.2)	16	15.3 (13.5-16.8)
14	66	19.2 (15.0-22.8)	11	20.2 (19.2-21.5)	18	16.1 (13.0-18.5)	8	16.6 (14.2-17.5)
17	34	19.6 (15.5-22.8)	3	20.6 (19.5-21.8)	—	—	—	—
20	8	18.8 (15.8-22.5)	1	20.8	—	—	—	—

TABLE 26 — *Effect of water volume on the growth of A. glabratus*

Population	Number of snails tested	Volume of water per snail in milliliters	Size (in millimeters) of snails on day after hatching						
			30	45	60	75	90	105	120
F . . .	10	300-350	7.0 (5.5-9.0)	—	9.8 (8.0-12.0)	—	13.2 (10.0-14.8)	—	13.9 (11.8-15.5)
	24 *	10,000-12,000	6.1 (4.0-8.0)	8.1 (6.5-11.2)	9.4 (8.2-11.0)	15.2 (10.2-18.5)	18.1 (14.0-21.5)	19.7 (17.0-22.8)	20.4 (16.8-24.8)
Fx . . .	10	300-350	8.3 (7.0-9.5)	—	9.6 (8.2-10.8)	—	10.5 (9.5-11.5)	—	11.9 (10.0-13.8)
	27 **	2,500-3,000	5.6 (5.0-6.5)	9.8 (6.8-12.0)	13.1 (11.0-16.8)	14.6 (12.5-18.8)	15.3 (12.8-19.5)	15.5 (13.0-19.8)	15.5 (13.5-20.2)
	24 **	10,000-12,000	5.4 (4.8-8.0)	10.2 (7.0-14.0)	13.1 (8.0-19.5)	14.8 (9.0-23.0)	17.0 (13.2-25.0)	17.8 (13.0-27.0)	18.1 (13.2-27.0)

* Introduced into aquaria on the 60th day after hatching.

** Introduced into aquaria on the 30th day after hatching.

TABLE 27 — *Comparative fecundity of A. glabratus maintained with and without oxygenating weeds*

Generation	Mode of maintenance	Number of snails at		Number of egg-clutches at		Number of egg-clutches laid by one snail in one day on month													
		Start	End	Start	End	1	2	3	4	5	6	7	8	9	10	11	12	13	14
F _{1x} . .	Paired	16	4	448	28	0.39	0.47	0.93	0.61	0.40	0.50	0.43	0.29	0.39	0.20	0.25	0.34	—	—
						0.18	0.48	0.45	0.30	0.31	0.33	0.18	0.14	0.15	0.27	0.42	0.20	—	—
	Singly	4	1	138	3	0.79	0.88	1.53	1.05	0.73	1.17	0.82	0.48	0.37	0.45	0.50	0.65	0.95	0.10
						0.16	0.67	0.57	0.75	0.43	0.66	0.37	0.32	0.34	0.42	0.48	0.47	0.38	0.03
F _{2x} . .	Paired	14	6	404	31	0.96	0.73	0.63	0.60	0.45	0.29	0.33	0.36	0.48	0.42	0.42	0.28	0.17	—
						0.43	0.42	0.25	0.34	0.24	0.24	0.38	0.40	0.34	0.17	0.28	0.14	0.07	—
	Singly	3	1	116	1	1.29	0.98	0.60	0.52	0.35	0.32	0.87	0.60	1.07	0.30	0.30	0.03	—	—
						0.40	0.42	0.78	0.55	0.33	0.08	0.05	0.02	0	0	0	0	0	0

TABLE 28 — *Effect of calcium on growth, fecundity and mortality rate in A. glabratus*

Month after first oviposition	+ CaCO ₃			— CaCO ₃		
	Mean length of shell in millimeters ¹	Mean egg mass per snail per day ¹	Percent of survivors in matures	Mean length of shell in millimeters	Mean egg mass per snail per day	Percent of survivors in matures
1	13.9	1.30	—	6.8	0.84	—
2	15.4	1.32	—	13.4	0.67	—
3	16.3	1.08	90.4	13.6	0.43	50.5
4	17.2	1.01	—	15.2	0.36	—
5	17.7	0.81	—	15.8	0.25	—
6	17.7	0.66	78.6	16.1	0.21	25.7
7	18.4	0.44	—	16.3	0.18	—
8	18.8	0.27	—	16.9	0.14	—
9	19.3	0.12	68.1	17.5	0.23	17.1

¹ Maintained singly.TABLE 29 — *Effect of calcium carbonate on the relationship between total weight and relative shell weight of A. glabratus (16-18 months old)*

Number of snails tested	CaCO ₃	Range of size in millimeters	Range of total weight in milligrams	Range of shell weight in milligrams	Range of shell percentage
13	With	19.0-22.8	505.7-996.1	190.7-362.7	31.0-43.5
8	Without	15.5-20.2	270.5-795.5	64.6-152.0	19.1-23.8

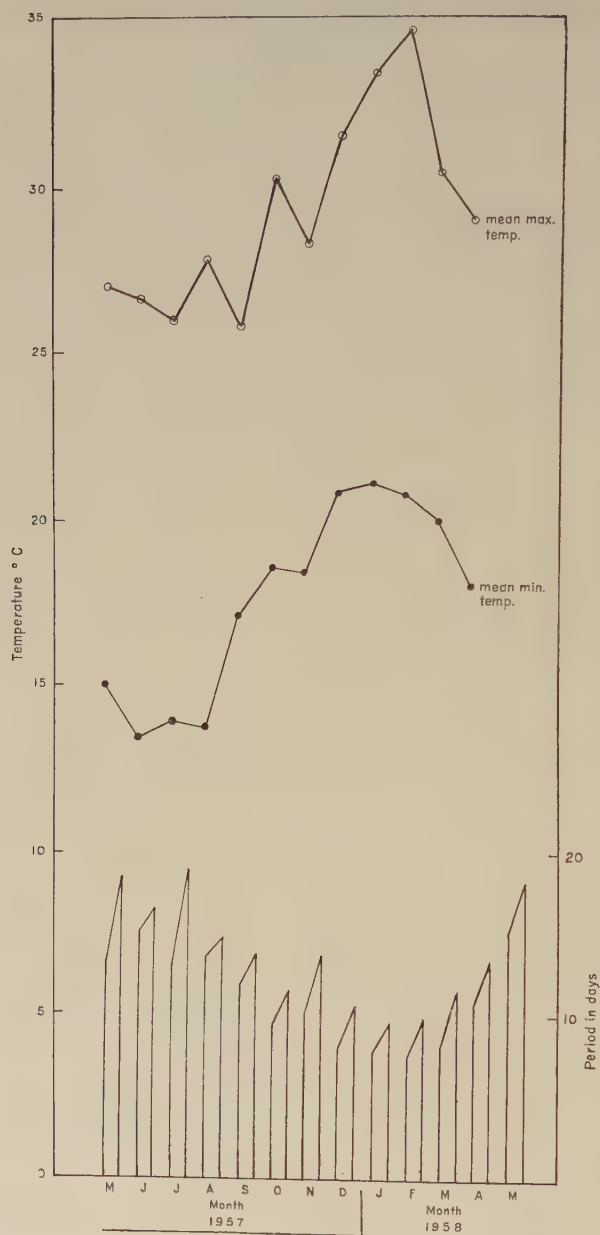


Fig. 1 — Effect of temperature variations on the incubation period of *A. glabratus* eggs

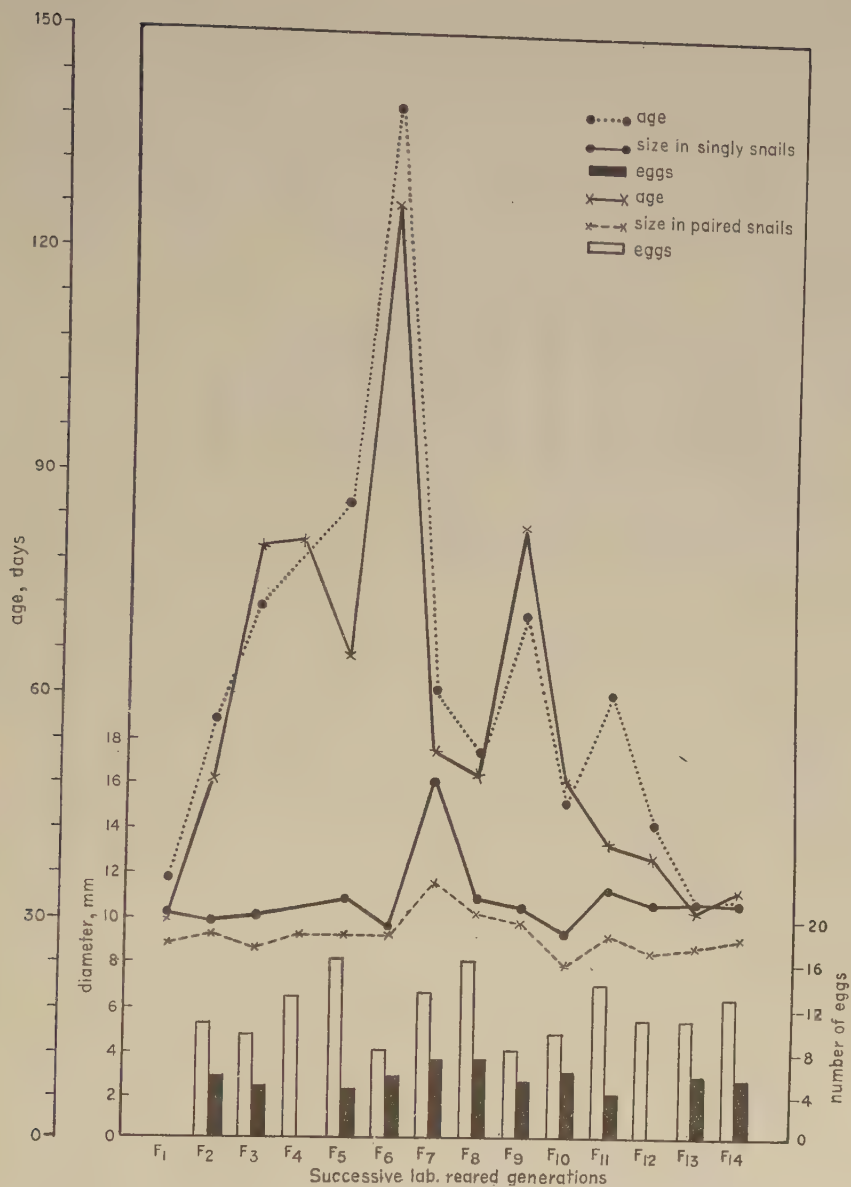


Fig. 2 — Showing the fluctuation in age, size and number of eggs laid by singly and mated *A. glabratus* on sexual maturity onset

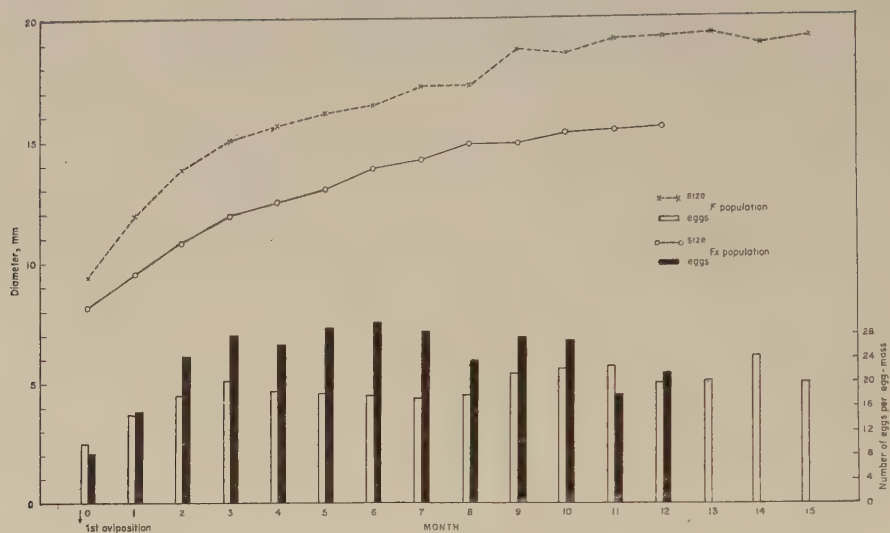


Fig. 3 — Showing the fluctuation in number of eggs per egg-mass in *A. glabratus*

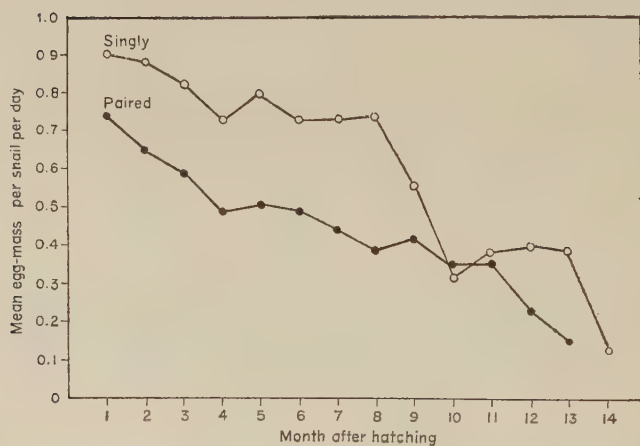


Fig. 4 — Showing the effect of increasing age on the fecundity of paired and singly *A. glabratus* (Fx population)

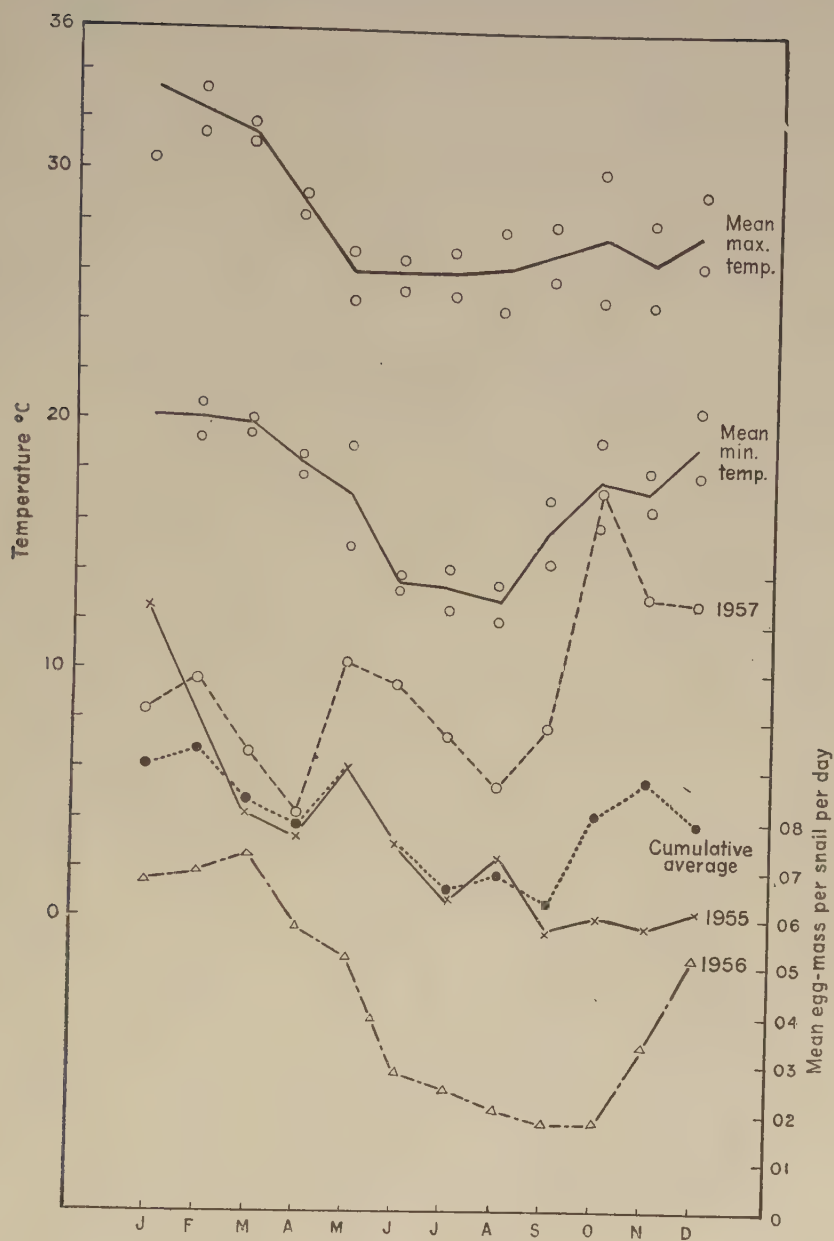


Fig. 5 — Showing the monthly variations in temperature and the fluctuation in snail fecundity

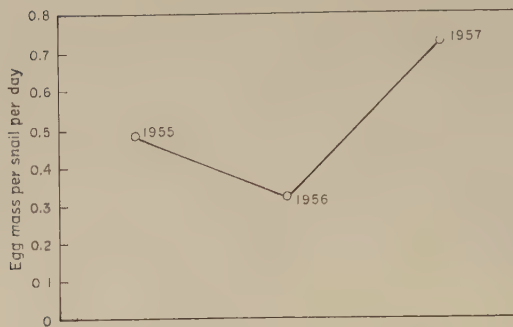


Fig. 6 — Showing the cyclic nature of reproductive activity in populations of intermingling old and young snails

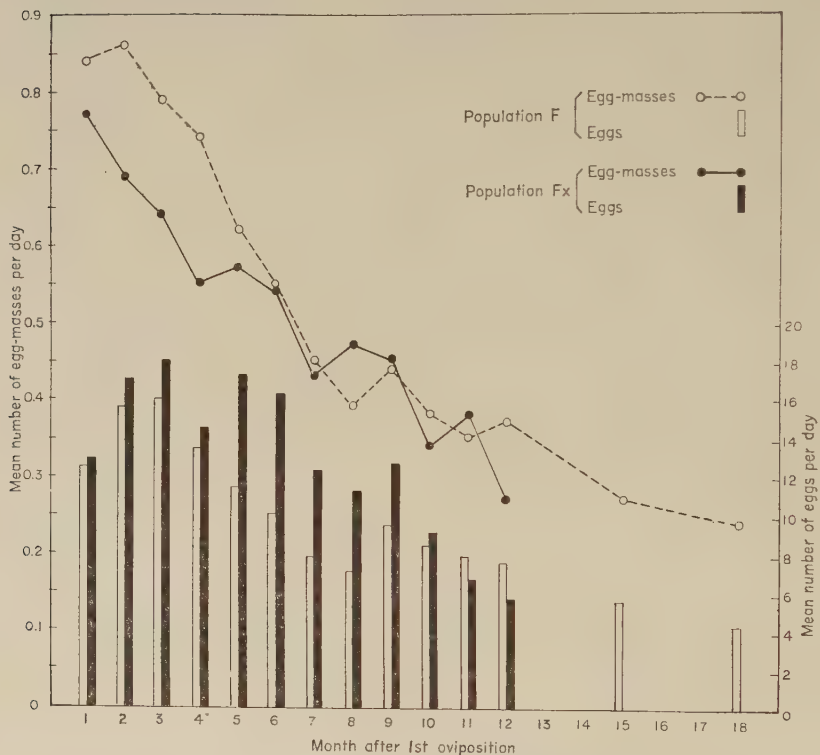


Fig. 7 — Showing monthly variations in reproductive potential of two populations of *A. glabratus*

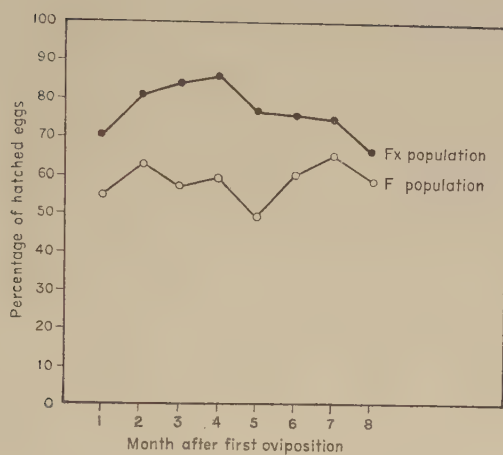


Fig. 8 — Showing the fluctuation in fertility of *A. glabratus* at increasing age

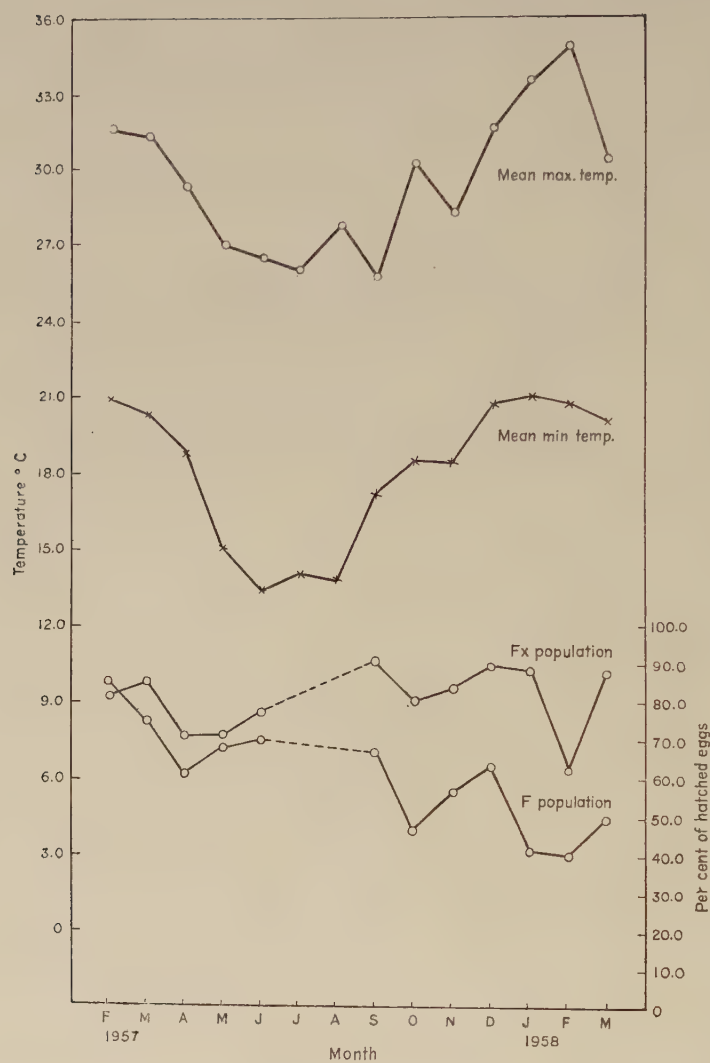


Fig. 9 — Showing seasonal variations of temperature and the fluctuation in hatchability of *A. glabratus* eggs

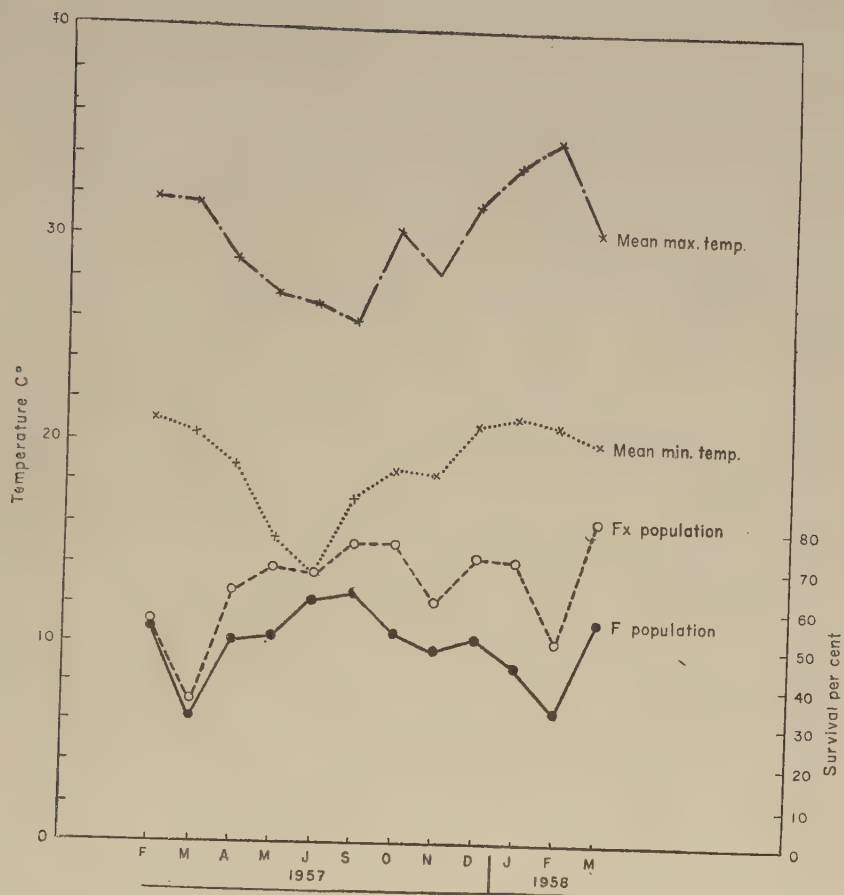


Fig. 10 — Showing seasonal variations in temperature and the fluctuation in infant survival from day 3 through 15 after hatching

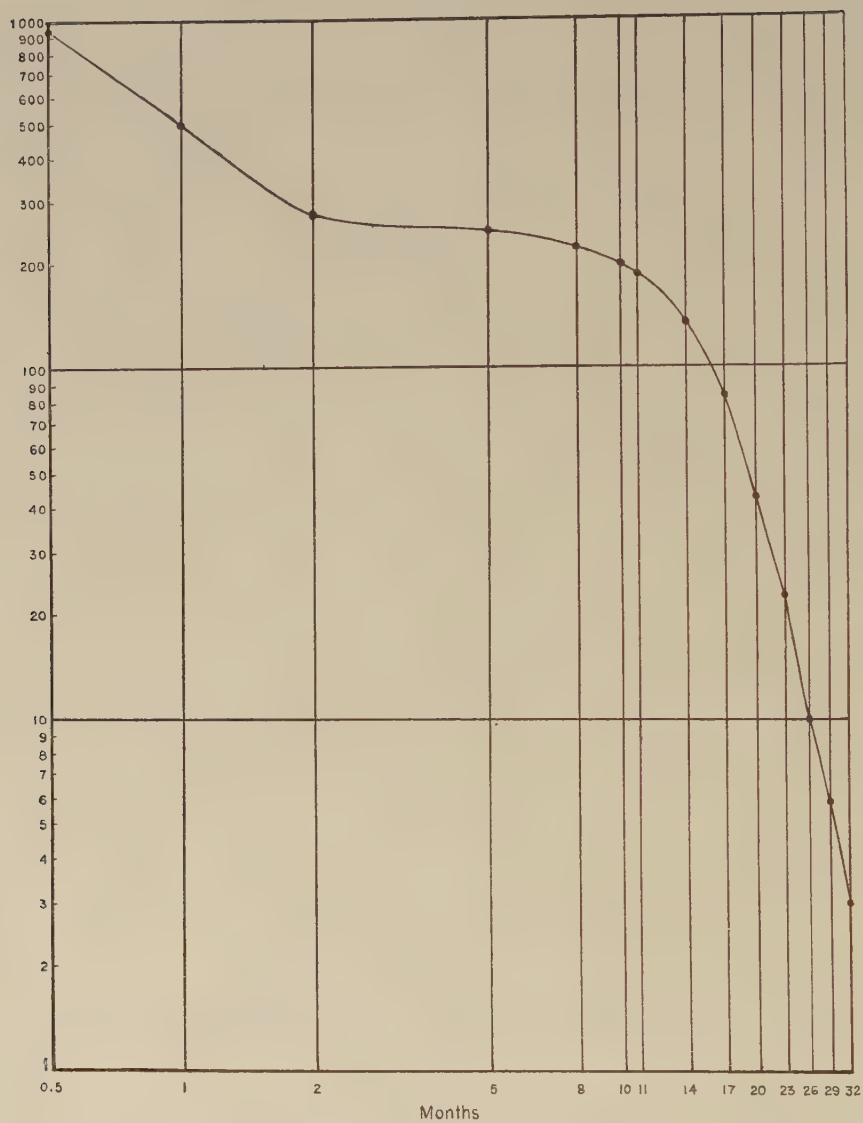


Fig. 11 — Number of survivors at the end of increasing periods of time in a theoretical group of 928 snails at hatching and in an actual group of 269 matures

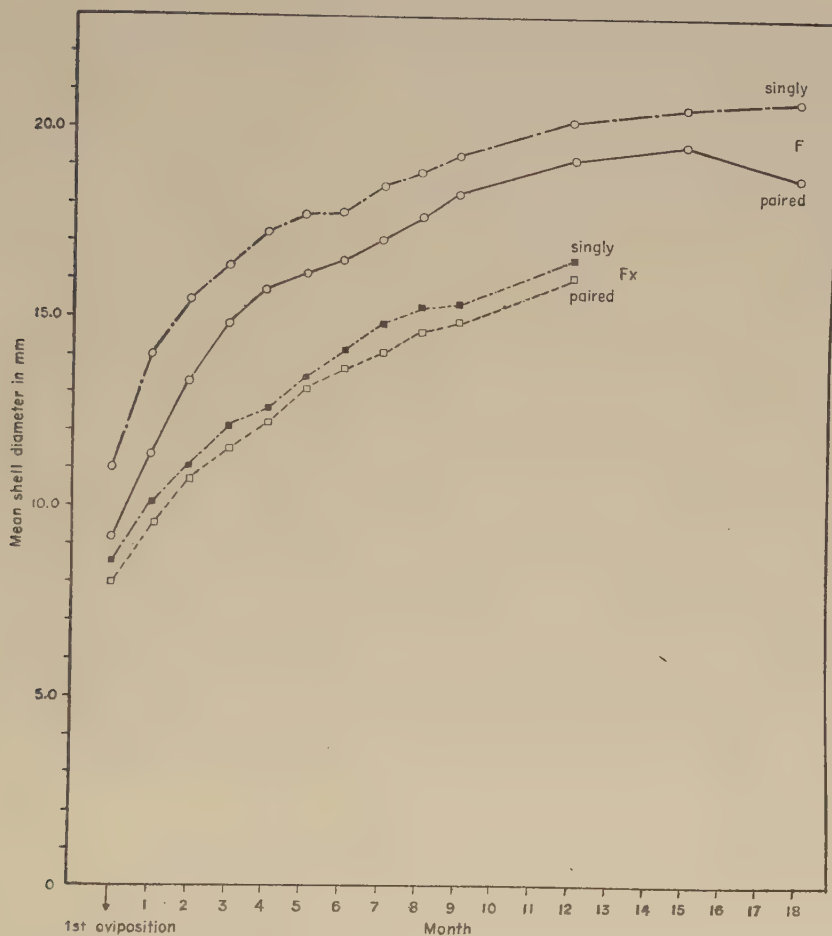


Fig. 12 — Mean shell measurements of *A. glabratus* in two populations maintained singly and paired in 300 to 350 ml of water per snail

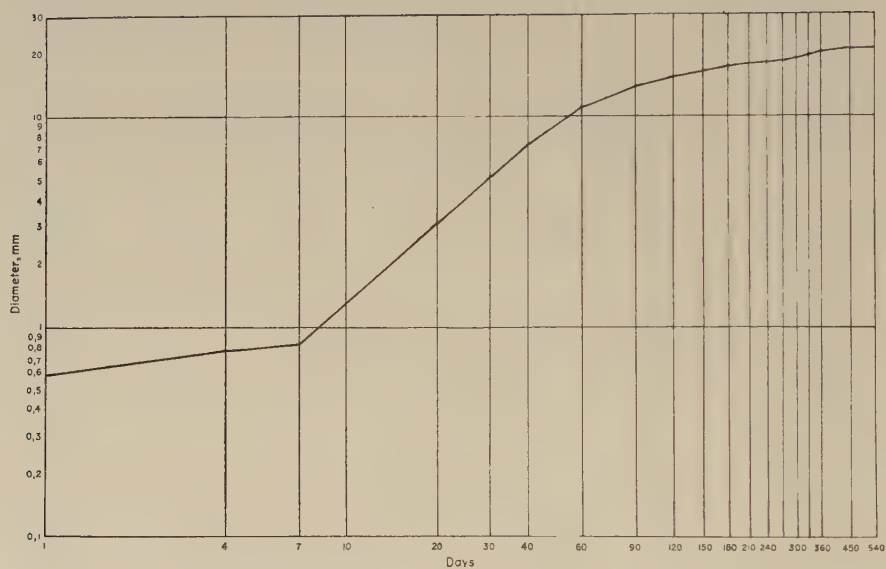


Fig. 13 — Mean shell measurements of *A. glabratus* from hatching through month 18

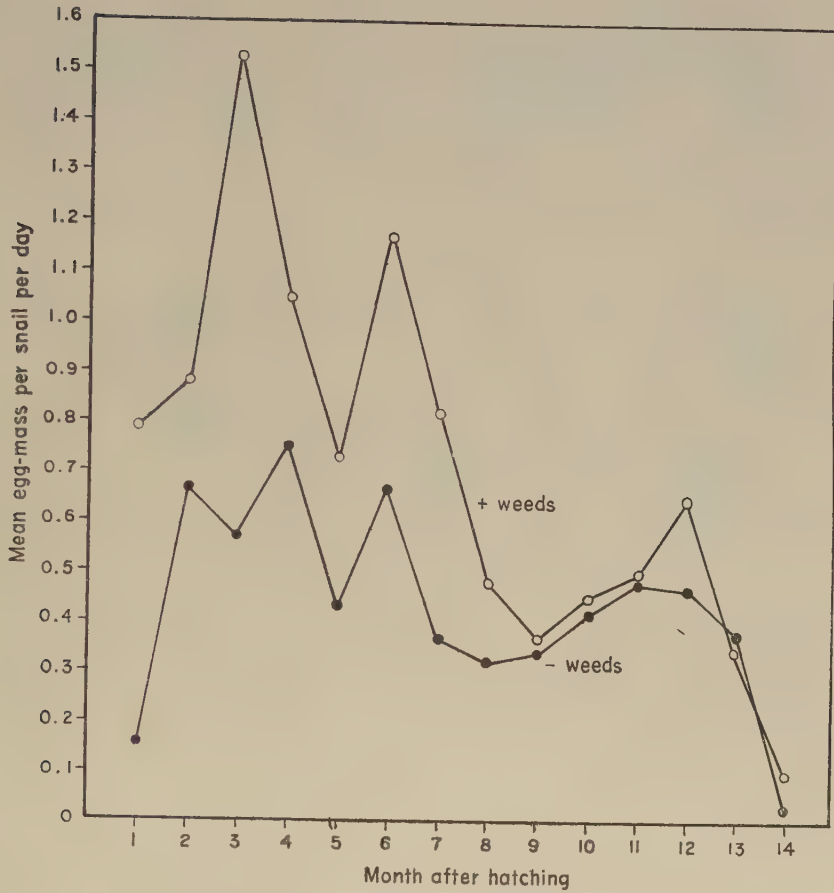


Fig. 14 — Showing the effect of oxygenating weeds on the fecundity of *A. glabratus*

LA BILHARZIOSE EN MAURITANIE. INFLUENCE DE L'ÉCONOMIE TRADITIONNELLE SUR LE CYCLE ÉPIDÉMIOLOGIQUE ET SUR LA RÉSISTANCE À L'INFESTATION

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APERÇU GÉOGRAPHIQUE

En 1958, la Mauritanie est sans doute encore le territoire le plus pauvre et le plus archaïque de toute l'Afrique Occidentale française.

Deux régions géographiques composent ce pays (voir carte jointe): 1) une zone sahélienne au sud, elle-même constituée par la vallée du Sénégal (Sahel sud) et par une bande de plaines plates dont la végétation arbustive ou herbacée est sous l'étroite dépendance du régime des pluies (Sahel nord); 2) une zone saharienne, formée de dunes ou de plaines arides et pierreuses dont la végétation demeure chétive et transitoire sous un climat sec, souvent brûlant, et des pluies irrégulières.

Dans ces zones, des massifs montagneux (Adrar, Tagant, Assaba) abritent de belles palmeraies, fertilisées par des pluies plus fréquentes et par les eaux drainées par les torrents, autour desquelles se sont édifiées un certain nombre d'agglomérations permanentes.

Dans l'ensemble, la Mauritanie est un pays aride, la plus grande partie de sa superficie étant située en zone saharienne. Le climat, bien que chaud, y est cependant salubre.

La zone sahélienne est marquée par une courte saison de pluies (juillet à octobre) et par une longue saison sèche (novembre à juin). La zone saharienne n'a pas de saison pluvieuse caractérisée; les pluies

y sont rares, les nuits tempérées; toutefois les écarts de température pendant le nycthémère y sont plus sensibles que dans le Sahel.



(D'après J. Richard-Molard)

STRUCTURE ETHNIQUE

La population de ce territoire est le fruit d'invasions successives.

Les Noirs qui peuplaient originellement toute la Mauritanie jusqu'en Adrar et au Tagant ont été rejetés vers la vallée du Sénégal par les Arabes et les Berbères; ils s'y sont maintenus sans peine et vivent en cultivateurs sédentaires. Ce sont surtout des Toucouleurs, des Sarakhollés, quelques Ouoloffs et quelques Bambaras.

Au nord de cette vallée, de l'Atlantique au Soudan et vers le nord jusqu'au Maroc, les Arabes règnent en maîtres. Ils forment la

majeure partie de la population, soit environ 470.000 âmes sur un total de 570.600 habitants.

Tandis que le pays noir se concentre dans des cantons administratifs dans la vallée du fleuve (Trarza, Brakna, Golgol), les collectivités arabes, partagées en tribus commandées par un chef ou un émir, vivent éparpillées dans les plaines sahéliennes ou sahariennes; elles pratiquent toutes le nomadisme.

Les Maures blancs y occupent une situation socialement dominante, en particulier les tribus guerrières (Hassans). Mais depuis quelques siècles, la pénétration de l'Islam y a ajouté la couche sociale des Marabouts; ceux-ci ne sont pas armés et leur situation est de ce fait plus précaire; mais leur influence reste grande en raison de leurs qualités de prédicateurs, de pédagogues ou de commerçants.

Riches ou pauvres, leucodermes ou mélanodermes, les tribus maures ont toutes la même structure interne: autour du chef gravitent la noblesse guerrière (Hassans) ou de robe (Marabouts), des tributaires (Haratins), asservis par la conquête et affranchis par la suite, des esclaves (ouvriers, domestiques, griots), enfin des hommes libres qui se sont volontairement intégrés dans la communauté.

A côté des Maures, les Peuls ou Foulbés forment une étrange minorité de 65.000 individus. Nomades de la brousse, on les rencontre surtout dans le Sahel. Ils vivent isolément dans la savane, par petites fractions conduites par un chef, et ne se regroupent autour des puits ou des rivières qu'à la fin de la saison sèche. C'est une population blanche d'apparence; mais un contact prolongé avec des populations de race noire, la pratique du servage, ont fini par déterminer la création de sous-races plus ou moins métissées qui vivent en semi-nomades aux confins de la vallée du fleuve.

GENRES DE VIE ET ÉCONOMIE

L'économie traditionnelle repose sur l'élevage, à un moindre degré sur la culture et sur les palmeraies. Elle conditionne deux genres de vie, celle des Nomades (Maures et Foulbés) et celle des sédentaires (Noirs du bord du fleuve, Berbères des palmeraies).

Chez les Nomades, l'élevage de type extensif domine toute la vie des tribus, car seul le bétail peut permettre à l'homme de tirer sa

subsistance de la maigre couverture végétale du sol. Déjà pauvre et saisonnière dans le Sahel, elle s'appauvrit d'autant plus que l'on remonte vers le nord et que l'on s'éloigne de l'Océan. Le Maure ou le Foulbé sont donc des pasteurs nomades dont l'existence est vouée à la recherche de pâturages pour faire paître leurs troupeaux: bovins, ovins, caprins, ânes et chameaux sont ainsi conduits à travers les friches suivant un rythme migratoire, en partie dicté par les exigences des saisons, en partie également par la tradition ou la fantaisie. Le cheptel faisant l'objet de ces transhumances est très prospère: 265.397 bovins; 2.301.006 ovins et caprins; 113.061 chameaux (en 1949, d'après les «Annexes Statistiques» des *Carnets d'Outremer*). Mais cet élevage important ne sert pour le moment qu'à couvrir les besoins immédiats des éleveurs, plus d'ailleurs sous forme de laitage que de viande.

L'habitat s'est adapté à cette vie itinérante: il est constitué par des tentes ou des cases hâtivement édifiées. Les tentes sombres, coniques, en poil de chameau, caractérisent les campements maures, tandis que les cases de paille hémisphériques, les *zéribas* de branches épineuses défendant les parcs de jeunes bêtes contre les attaques d'animaux sauvages, dénoncent la présence des campements peuls.

La traite quotidienne du lait est faite par les femmes, et le lait caillé plus ou moins coupé d'eau (*z'rick*), recueilli dans des callebasses, constitue l'essentiel de l'alimentation des Peuls qui ne songent pas, en général, à consommer la chair de leur bétail, à l'exception peut-être du mouton. Chez les Maures, le lait frais, la viande grillée en plein air (*méchoui*) ou en sauce figurent à tous les repas.

Les oasis sont les points fixes de cette vie nomade où se rencontrent marchés, entrepôts et centres de ravitaillement. Sans être comparables à celles d'Afrique du Nord, les palmeraies forment néanmoins, avec leur végétation étagée, les cultures de légumes, de tabac, de henné, les champs de mil ou de blé, des petits centres agricoles dont la population sédentaire tire tout ce qui peut être nécessaire à son existence.

Les terres basses du Sénégal, occupées par les Noirs sédentaires, ont une destinée totalement différente. Régulièrement inondées lors des crues annuelles, elles sont surtout cultivées en mil. De Boghé jusqu'au Soudan, les terres arrosées du Brakna, du Gorgol et du Guidimaka font l'objet de deux récoltes; de février à avril, celle de

la saison sèche ou de décrue (mil oualo); d'octobre à décembre, celle de l'hivernage (mil dieri), effectuée sur les terrains plus élevés, en bordure de ceux envahis par les eaux et arrosés seulement par les pluies.

Dans certaines régions du Sahel (Aleg à Nema), partout où la configuration du terrain permet de retenir les eaux de surface pendant 2 ou 3 semaines, les Nomades font des cultures vivrières (légumes, riz, mil oualo), qui sont souvent confiées à des agriculteurs sédentaires. A l'inverse, partout où l'agriculture occupe la première place et où l'élevage ne joue qu'un rôle accessoire, on fait souvent appel à des Peuls pour assumer la garde du bétail.

INCIDENCES DE CES TYPES D'ÉCONOMIE SUR L'ÉPIDÉMIOLOGIE DE LA BILHARZIOSE

La bilharziose due au *Schistosoma haematobium* semble largement répandue en Mauritanie.

Qu'elle existe dans les régions occupées par les sédentaires n'a pas lieu de surprendre. Les rapides indications que nous venons de donner sur les cultures vivrières (mil oualo) et les inondations annuelles montrent à l'évidence que les conditions nécessaires au déroulement du cycle épidémiologique se trouvent réunies: fréquentation habituelle des mares ou des champs de cultures inondées, présence maintes fois observée de bulins, hôtes intermédiaires, chaleur favorisant la concentration des cercaires dans les marigots en voie d'assèchement.

De fait, les premières prospections faites dans la vallée mauritanienne du Sénégal (*Notes sur la prospection de la Bilharziose dans la vallée du Sénégal*, février 1958) ont fait apparaître un taux assez élevé de porteurs d'œufs de *S. haematobium* (Diandoli: 56 pour-cent; Vindou Bosséabé: 51 pour-cent); et l'examen de mares temporaires dans les mêmes régions (Vindou Owro; Vindou Bérobé; Vindou Bosséabé; Vindou Walo) a permis de déceler la présence de *Bulinus guernei* et *B. forskalii*, tous deux vecteurs de la schistosomose à *haematobium*. Des élevages de ces mollusques faits au laboratoire ont montré la grande réceptivité de *B. guernei* aux infestations expérimentales.

Mais, si les conditions de contamination et les poussées épidémiques obéissent ici à des impératifs connus, il n'en est pas de même chez les Nomades, où la bilharziose vésicale existe également dans un pourcentage de cas assez important.

Les tribus, en effet, ne séjournent jamais très longtemps au même endroit: les limites d'épuisement du sol, l'assèchement plus ou moins rapide des points d'eau conditionnent la durée de leurs campements, de même que la rentabilité de terres voisines et la proximité d'un puits ou marigot détermineront le choix d'un nouvel emplacement.

La fréquentation des marigots où vivent les bulins est donc essentiellement provisoire, mais elle est aussi cyclique. Les porteurs d'œufs de schistosome promènent le germe sur un vaste périple, mais reviennent périodiquement aux mêmes gîtes; l'implantation de la bilharziose dans ces gîtes et leur transformation en foyers d'endémie n'a pu se réaliser qu'en raison de la périodicité de ces cycles migratoires, périodicité qui s'étale sur un assez grand nombre d'années. À ce facteur d'implantation s'en ajoute un second, non moins important: c'est la fréquentation massive des mares pendant la saison sèche par les enfants et les jeunes adolescents qui s'ébattent tout le jour dans l'eau tiédie et constituent presque exclusivement les réservoirs de parasites. C'est en mars, avril, mai et juin, lorsque les cercaires se concentrent dans des collections d'eau de plus en plus réduites, que les infestations deviennent les plus massives. Les maxima d'hématuries s'observent chaque année à la fin de cette saison sèche (mai, juin); elles s'atténuent progressivement pendant la saison pluvieuse et le début de la période sèche (octobre, novembre, décembre) en raison de la température assez basse, même dans la journée. Dans ces populations xérophiles, la femme n'intervient guère comme réservoir de germe; ses vêtements sombres, la rareté et l'éloignement de l'eau relèguent au second plan les importantes lessives qui, dans d'autres foyers, sont à l'origine de tant d'infestations.

Dans ces conditions, la bilharziose des Nomades s'avère donc épidémiologiquement assez différente de celle observée dans les foyers fixes dont le caractère endémique est dû à la permanence des contacts entre les facteurs d'évolution du ver. L'interruption des rapports entre le mollusque et l'homme pour des périodes assez longues et la reprise rapide du développement du ver dès que ces contacts se rétablissent impliquent nécessairement, semble-t-il, la possibilité pour le germe

d'entrer aisément en diapause chez ses différents hôtes. Dans ce cycle épidémiologique à éclipses, le schistosome s'est adapté à un type d'évolution particulier à certains trématodes, parasites d'animaux migrants, dont le développement supporte chez le mollusque des périodes de latence que ne peuvent atteindre ni les rigueurs du climat, ni les excès de la température.

La bilharziose bovine s'est-elle introduite dans les mêmes conditions? Signalons simplement que les troupeaux fréquentent les marigots hébergeant les vecteurs communs aux deux schistosomoses suivant les mêmes alternances que les porteurs humains, et que l'on observe fréquemment chez les bovidés des diarrhées sanglantes dont certaines pourraient être en rapport avec une infestation bilharzienne.

La gravité de la bilharziose vésicale est souvent contestée. Elle est évidemment fonction des taux d'infestation. Maladie de l'enfance, elle disparaît le plus souvent lorsqu'il devient adulte et ne fréquente que de loin en loin les lieux d'infestation. Associée à d'autres helminthiases, on sait qu'elle peut être à l'origine d'anémies graves surtout chez les indigents, les sous-alimentés. Il ne nous appartient pas d'aborder ici le problème de la nutrition sinon pour rappeler qu'envisagée sous l'angle qualitatif plus que quantitatif, la malnutrition inhibe la résistance de l'individu à un grand nombre de maladies infectieuses ou parasitaires.

Dans l'exemple qui nous retient ici, nous avons pu remarquer que, chez les Maures, les jeunes enfants nourris presque exclusivement de lait et de viande présentaient parfois une résistance remarquable aux infestations vermineuses, malgré l'insuffisance des rations alimentaires. Sans nul doute, cet apport quotidien de protides et de fer leur permet de lutter efficacement contre les déficiences consécutives à un parasitisme élevé et surtout de réparer leur anémie malgré des hématuries importantes.

Elargissant cet aspect du problème, J. de Castro (1956) a constaté que, sur tous les continents encore sous-développés, les populations pastorales, grandes consommatrices de produits d'origine animale (Berbères du Sahara, Maures, Peuls, Nègres soudanais faisant de l'élevage dans les savanes africaines, Pasteurs du Haut Nil, Massai du Kenya, habitants du Penjab) présentaient, en dépit de leur sobriété, une résistance organique infiniment supérieure à celles des populations

voisines, soumises à un régime également fruste mais uniquement végétarien.

On admet aujourd'hui que les sols tropicaux et subtropicaux sont peu propices à la production de produits animaux, sources essentielles de protéines et de fer assimilables; ceci résulterait d'une part des grandes superficies envahies par la forêt vierge et retirées à l'élevage, d'autre part de la pauvreté des terres en produits azotés dont une partie se solubilise encore et disparaît sous les eaux torrentielles des averses tropicales.

C'est pourquoi, en pays tropicaux où la forme d'anémie la plus répandue est à la fois d'origine alimentaire (hypoprotéïnémie) et parasitaire (maladies vermineuses), le problème de la compensation des carences organiques par un régime alimentaire approprié semble loin d'être résolu.

CONCLUSIONS ET RÉSUMÉ

Nous avons tenté d'examiner dans ce travail comment la bilharziose, qui s'observe en général lorsque des individus sédentaires ont des contacts permanents avec l'eau parasitée par les mollusques, a pu se maintenir chez des populations en déplacements constants et particulièrement xérophiles.

Les points d'eau sont en effet fort rares dans les plaines sahéliennes ou sahariennes; les adultes n'en usent que fort peu et la consomment le plus souvent chaude ou bouillie (thé). Seuls les enfants sont soumis pendant la saison d'été à des infestations massives, mais essentiellement temporaires. Ils suffisent néanmoins à jouer le rôle de réservoirs de virus, et le parasite semble l'être adapté aux longues périodes de latence pendant lesquelles les contacts sont rompus entre le mollusque et l'hôte définitif humain.

Nous nous trouvons ici devant un type d'épidémiologie assez inhabituel chez les schistosomés et qui s'apparente au parasitisme des espèces animales migratrices.

La résistance organique et la vivacité présentées par les sujets atteints d'infestations sévères, chez les tribus maures ou foubés, nous semblent être en rapport avec le régime alimentaire, riche en protéines animales, qu'ils consomment quotidiennement. Le problème de la nutrition, mis au premier plan en A.O.F. par les Services d'Hygiène, a fait apparaître combien la malnutrition, plus encore que la sous-alimentation pouvaient diminuer les possibilités de résistance des individus. Aussi semble-t-il de plus en plus que, parallèlement aux recherches sur la prophylaxie dont on connaît les difficultés, il faille s'orienter vers des études qualitatives sur la nutrition dont certains principes s'avèrent souvent comme les meilleurs agents neutralisants de nombreux germes infectieux ou parasitaires.

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GENERAL DISCUSSION

DR. FELIX PIFANO (Venezuela) — En el diagnóstico inmunológico de la schistosomiasis mansonii en Venezuela, nosotros empleamos de rutinas las siguientes pruebas biológicas:

1) Intradermorreacciones de lectura inmediata, empleando como antígeno extractos de vermes adultos de *Schistosoma mansonii*;

2) Reacción de desviación del complemento según Fairley, empleando como antígeno extracto alcohólico de hepato-páncreas de *Australorbis glabratus* (el huesped intermediario en Venezuela) infectados con cercarias de *Schistosoma mansonii*;

3) Acción de los inmuno-sueros sobre las cercarias de *S. mansonii* (cercaria reacción de Vogel Minning).

En 400 casos de schistosomiasis mansonii de diagnosticados parasitológicamente mediante el examen de heces o por biopsia rectoscópica, la intradermorreacción ha sido positiva en el 95.5 por ciento de los casos.

En 497 casos de schistosomiasis mansonii parasitológicamente comprobados, la reacción de desviación del complemento según Fairley ha sido positiva en el 74 por ciento de los casos.

En 1,380 casos de schistosomiasis mansonii diagnosticados parasitológicamente, la cercaria reacción fué positiva en el 78 por ciento de los casos.

En casos observados durante la fase de invasión de la schistosomiasis mansonii, en los cuales se practicó simultánea y periódicamente a partir de los baños infectantes, el examen coprológico, la intradermorreacción, la reacción de fijación del complemento, y la cercaria reacción, la positividad de la cercaria reacción comenzó a partir de la 4.^a semana de los baños infectantes precediendo a la positividad de las otras reacciones y a la aparición de los huevos en las heces.

Sobre un total de 200 casos de schistosomiasis sometidos a la medicación antimonial (3 series de tartrato doble de antimo-

nio y potasio de 1.50 g por serie y con intervalos de 2 semanas entre cada serie; tratamientos intensivos de 3 días con 0.60 g de tartrato doble de antimonio y sodio; 3 series de Fuadina con intervalos de 1 semana entre cada serie), la intradermorreacción mantuvo su positividad en el 80 por ciento de los casos, la reacción de desviación del complemento se negativizó en el 32.35 por ciento de los casos y la cercaria reacción en el 50 por ciento de los casos. Estas reacciones de control se practicaron 3 a 6 meses después de haber concluido el tratamiento.

DR. A. H. MOUSA (Egypt) — In relation to what Dr. Prates concluded for the frequency of the malignancy in bilharzias cases, in our Country no single case was recorded in pure cases of hepatic bilharziasis. It were only met with in cases showing portal cirrhosis as well. It is most probable that the cases of Dr. Prates are survivals as our cases rarely survive after the thirties.

Dr. Augustine confined the etiology of hepatic bilharziasis to worms only while these are responsible for the stem type while the ova is responsible for the diffuse type.

As regards Dr. Silva, he is treating his cases slowly, as we had that tendency to lower the incidence of hepatic shift responsible for the aggravation of the disease.

DR. J. RODRIGUES DA SILVA (Brasil) — O Dr. Mousa mencionou a possibilidade de agravação da doença, por efeito do tratamento específico. Este ângulo do problema tem merecido amplas considerações e minuciosos estudos dos pesquisadores brasileiros. A impressão actual é de que apenas em casos com lesões hepáticas avançadas o tratamento específico poderia condicionar agravamento da hepatopatia, por acção dos vermes mortos, arrastados para o interior do fígado. Isto condicionaria processos isquémicos e acentuaria a fibrose hepática preexistente. Alguns trabalhos experimentais sobre a matéria, realizados em animais de pequeno porte, podem, entretanto, ser aplicados ao homem.

Por outro lado, as observações de casos clínicos humanos submetidos à estibioterapia, que se contam por vários milhares, não têm servido de suporte aos referidos trabalhos experimentais. Pelo contrá-

rio, o que se observa, regra geral, é uma atenuação das manifestações clínicas da doença após o tratamento específico. Observações feitas no Brasil, por sua vez, tendem à conclusão de que a incidência de formas graves da doença tem sido reduzida, como resultado do emprego da terapêutica específica.

DR. EMMANUEL DIAS (Brasil) — Como contribuição ao tema «luta biológica contra os planorbídeos», daremos um informe acerca das investigações que vimos fazendo em Bambuí, Brasil.

O nosso maior foco observado, n.º 27 (cuja observação foi publicada nas *Memórias do Instituto Oswaldo Cruz*, 52: 315-327, 1954, e em *O Hospital*, 47: 543-555, 1955), foi primeiramente tratado com culturas de *Bacillus pinottii* em Abril de 1953.

Seguiu-se grande mortandade de caramujos, sendo o número máximo de mortos recolhido 4 meses depois. Numa última contagem, feita em fins de 1957, foram apenas encontrados 150 *A. glabratus* vivos e cerca de 1500 mortos.

A mortandade foi lenta, progressiva e específica. A enorme população a princípio existente foi quase totalmente destruída e, o que parece mais importante, não conseguiu recuperar-se, devido, possivelmente, à permanência de uma acção moluscocida residual pelos agentes vivos empregados, que já vêm durando há vários anos.

DR. EMILE A. MALEK (Sudan) — The results arrived at in the report by D. L. Augustine, E. Chernin, C. Pan and E. Michelson on fundamental studies for the biological control of schistosome-bearing snails are very interesting and should be encouraged and continued because of their importance in a better understanding of the biology of the snail host. However, we should not be very hopeful as to their effect in the biological control of schistosomiasis intermediate hosts.

It is not very likely that the predators reported here would play a significant role in the control of the snail. More field studies should be carried out on the role which *Marisa* plays in controlling populations of *Australorbis glabratus*. There may be some hope from the results of investigations on bacteria, fungi and viruses in controlling snails.

Information on the biology of the snails are lacking and are essential and the aim should be directed towards increasing our knowledge

of the epidemiology and transmission of the disease and towards a proper application of molluscicides to achieve effectiveness and economy.

Schistosomiasis has been compared to malaria. One, however, has to remember the fundamental difference between the two diseases. The mosquito comes to man and infects him, but man goes to the snail habitats, infects the snails and becomes infected. I am stressing here the cultural aspect of schistosomiasis and that attention should also be given, in endemic areas, to the education of the individuals and to a proper sanitation.

3RD SESSION: Tuesday, September 9th — 11:00 to 12:30 a.m. Room D

The Chairman of this meeting was Dr. Thomas Weller and the Rapporteur was Dr. F. Simões Barbosa. The Secretary was Dr. M. da Costa Faro. The following papers were presented and, as some Co-Rapporteurs were not present, the Chairman decided to call the authors of the free communications offered to this Subsection.

OBSERVAÇÕES ECOLÓGICAS SOBRE O *AUSTRALORBIS*
GLABRATUS EM BELO HORIZONTE (MINAS GERAIS).
BRASIL: I. DENSIDADE E VITALIDADE¹

ROBERTO MILWARD DE ANDRADE

e

JOSÉ RABELO DE FREITAS

Departamento Nacional de Endemias Rurais
Brasil

RESUMO

Os AA., estudando a ecologia do *Australorbis glabratus* em uma área da Represa da Pampulha, tecem considerações sobre o valor da determinação de alguns factores que condicionam o *habitat* dos planorbídeos, realçam a importância desses estudos e apresentam o método adoptado para a colecta dos moluscos. Descrevem ainda, sucintamente, as colecções hídricas estudadas e apresentam os aspectos fisio e fitográficos que serviram para distinguir ecológicamente os biótipos estudados.

Analisando os dados colhidos em 60 biótipos («estações») durante 18 meses consecutivos (Novembro, 1955 — Abril, 1957), verificaram que a área estudada apresentou condições favoráveis para os caramujos apenas no início das pesquisas, isto é, no fim do Estio de 1955.

¹ Trabalho do Departamento Nacional de Endemias Rurais.

Os dados de densidade e vitalidade sugerem que a procriação se dá no período de Estio e o crescimento no período chuvoso, época essa que coincide com uma grande queda na densidade. Verificaram também que a limpeza nos cursos de água (valas e córregos) ora concorre para eliminar os caramujos, ora cria condições favoráveis ao seu desenvolvimento. Concluem finalmente que, na área estudada (com aproximadamente 2000 m de extensão), os locais que apresentam condições necessárias para a manutenção de uma população de planorbídeos em equilíbrio ecológico não ultrapassariam 1 por cento de toda a área. Em suma, o *habitat* do *Australorbis glabratus* é muito restrito, mas o poder de dispersão da espécie é extraordinário.

SOBRE UM INQUÉRITO PARASITOLÓGICO AOS COLONOS EUROPEUS ESTABELECIDOS NO ESQUEMA DE IRRIGAÇÃO DO VALE DO RIO LIMPOPO

TITO DE MORAIS

Instituto de Investigação Médica de Moçambique,
Secção de Bilharziose

A elevada incidência de bilharziose e outras parasitoses intestinais entre os africanos residentes na área do vale do rio Limpopo, verificada aquando do inquérito realizado pela missão do Instituto de Medicina Tropical de Lisboa, em 1954, levou-nos a averiguar até que ponto os colonos europeus ali estabelecidos teriam sido afectados, ao fim de um ano, por essas endemias.

Realizámos então, entre Abril e Junho de 1956, um inquérito parasitológico entre todos os habitantes europeus da área de colonização, o que nos permitiu ficar com uma ideia da situação desses colonos, tendo em vista um conhecimento prévio que nos sirva de base aos trabalhos de pesquisa de métodos de *contrôle* a aplicar na região que escolhemos como zona-piloto para as nossas experiências de bilharziose.

MATERIAL E MÉTODOS

O inquérito baseou-se no exame de 558 indivíduos de ambos os sexos, de idades variando entre os 6 meses e os 75 anos, de raça europeia, por meio de análises de sangue, urinas e fezes e palpação do baço e fígado.

Os exames de urinas e de fezes eram feitos pela técnica de sedimentação simples de Hoffman, Pons e Jenner. O exame de sangue, por gota espessa e esfregaço de sangue colhido por punção digital.



Fig. 1 — Divisão administrativa da província de Moçambique

A palpação do baço e fígado, com o examinado de pé, em ligeira flexão anterior do tronco. A esplenomegalia era avaliada pelo método recomendado pela Organização Mundial de Saúde e o grau de hepa-

tomegalia medido por dedos travessos abaixo do rebordo costal, numa linha imaginária ligando o mamilo direito com o umbigo.

RESULTADOS

Nos quadros 1, 2 e 3 apresentamos os índices de prevalência das diferentes parasitoses por sexos e grupos de idade.

Tendo em consideração que se tratava de indivíduos com pouco mais de um ano de residência em África, tendo vindo de territórios onde não existe bilharziose, a presença de 2,86 por cento de casos de bilharziose vesical entre os 558 indivíduos examinados é um indício seguro de que a região se tornará, com o tempo, um local de alta prevalência desta endemia entre os colonos, se não forem aplicadas medidas de *contrôle* eficientes, que se tornam urgentes.

Dos parasitas intestinais diagnosticados, o *Trichiurus* e o *Ascaris* foram os que apresentaram maior índice de prevalência, sendo, no entanto, provável que os doentes já fossem portadores deles antes da sua chegada ao Limpopo, visto virem de zonas onde estas doenças são vulgares (Alentejo).

De uma forma geral, a prevalência de parasitas é reduzida, o que nos permitirá ter uma boa referência para o futuro.

No que respeita à prevalência de esplenomegalia e hepatomegalia, podemos verificar pelos quadros 4 a 6 que os seus índices são insignificantes, como seria de esperar numa população que só recentemente tem sido exposta à acção dos seus principais factores determinantes.

Tendo o Instituto de Investigação Médica em vista estabelecer uma estação de estudo no Guijá, com o objectivo de servir como zona-piloto para trabalhos de investigação sobre bilharziose, parasitoses intestinais, malária e adaptação climática do trabalhador europeu em África, o inquérito parasitológico realizado afigura-se-nos de grande interesse como base para comparação com os dados que futuramente esta estação de estudo vá obtendo.

No que respeita à bilharziose, os estudos a realizar vão incidir especialmente sobre a ecologia e biologia dos moluscos vectores, bem como sobre os aspectos clínicos e sociais da doença.

QUADRO 1 — *Machos*

Grupos etários	Exames	B. vesic.		Ascaris		Trico.		Coli		Iodam.		Strongyl.		Lambia		Enter.		Tonla		Ancil.		Malária	
		Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem
0-4 . .	28	0	—	2	7,14	2	7,14	1	3,57	0	—	0	—	2	7,14	0	—	0	—	0	—	0	—
5-9 . .	27	3	11,11	2	7,40	4	14,81	6	22,22	1	3,70	0	—	0	—	3	11,11	0	—	0	—	0	—
10-14 . .	33	2	6,06	2	6,06	0	—	7	21,21	0	—	0	—	0	—	1	3,03	1	3,03	1	3,03	0	—
15-19 . .	42	2	4,76	0	—	1	2,35	5	11,90	0	—	0	—	0	—	1	2,35	0	—	1	2,35	0	—
+ 20 . .	217	7	3,22	14	6,45	12	5,52	18	8,29	4	1,84	1	0,46	0	—	5	2,30	0	—	1	0,46	0	—
Totais . .	347	14	4,03	20	5,76	19	5,47	37	10,66	5	1,44	1	0,28	2	0,57	10	2,88	1	0,28	3	0,86	0	—

QUADRO 2 — *Fêmeas*

Grupos etários	Exames	B. vesic.		Ascaris		Trico.		Coli		Iodam.		Enter.		Lambia		Ancil.		Malária	
		Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem
2-1 . .	24	0	—	1	4,16	0	—	1	4,16	0	—	0	—	0	—	0	—	0	—
5-9 . .	22	1	4,54	2	9,09	0	—	1	4,54	0	—	0	—	0	—	0	—	0	—
10-14 . .	22	0	—	3	13,63	2	9,09	4	18,18	1	4,54	1	4,54	1	4,54	0	—	0	—
15-19 . .	14	0	—	1	7,14	1	7,14	1	7,14	0	—	2	14,28	0	—	0	—	0	—
+ 20 . .	129	1	0,77	5	3,87	11	8,52	15	11,62	2	1,55	4	3,10	0	—	2	1,55	0	—
Totais . .	211	2	0,94	12	5,68	14	6,66	22	10,42	3	1,42	7	3,31	1	0,47	2	0,94	0	—

QUADRO 3 — Totais

Grupos etários	Exames	B. vesic.		Ascariis		Coli		Iodam.		Strongyl.		Lambdia		Enter.		Tenia		Trico.		Ancil.		Malaria	
		Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem
2-4. . . .	52	0	—	3	5,76	2	3,84	0	—	0	—	2	3,84	0	—	0	—	2	3,84	0	—	0	—
5-9. . . .	49	4	8,16	4	8,16	7	14,28	1	2,04	0	—	0	—	3	6,12	0	—	4	8,16	0	—	0	—
10-14. . . .	55	2	3,63	5	9,09	11	20	1	1,81	0	—	1	1,81	2	3,63	1	1,81	2	3,63	1	1,81	0	—
15-19. . . .	56	2	3,57	1	1,78	6	10,71	0	—	0	—	0	—	3	5,35	0	—	2	3,57	1	1,78	0	—
+ 20. . . .	346	8	2,31	19	5,49	33	9,53	6	1,73	1	0,28	0	—	9	2,60	0	—	23	6,64	3	0,86	0	—
Totais . . .	558	16	2,86	32	5,73	59	10,57	8	1,43	1	0,17	3	0,53	17	3,04	1	0,17	33	5,91	5	0,89	0	—

QUADRO 4 — *Machos*

Grupos etários	Exames	Esplenomegalia						Hepatomegalia										Hepatosplenomegalia							
		I		II		III		IV		Total		1		2		3		4		5		Total		Casos	Porcentagem
		Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem				
2-4 . . .	28	1	3,57	—	—	—	—	—	—	1	3,57	—	—	—	—	—	—	—	—	—	—	—	1	3,57	—
5-9 . . .	27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
10-14 . . .	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
15-19 . . .	42	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
+ 20 . . .	217	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Totais . . .	347	1	0,57	—	—	—	—	—	—	1	0,57	—	—	—	—	—	—	—	—	—	—	—	3	0,86	—

QUADRO 5 — *Fêmeas*

Grupos etários	Exames	Esplenomegalia						Hepatomegalia						Hepatosplenomegalia									
		I		II		III		IV		Total		1		2		3		4		5		Total	
		Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem	Casos	Porcentagem
2-4	24	1	4,16	—	—	—	—	—	—	1	4,16	1	4,16	—	—	—	—	—	—	—	—	1	4,16
5-9	22	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10-14	22	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15-19	14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
+ 20	129	—	—	—	—	—	—	—	—	—	—	4	3,1	—	—	1	0,77	—	—	—	—	5	3,87
Totais	211	1	0,47	—	—	—	—	—	—	1	0,47	5	2,36	—	—	1	0,47	—	—	—	—	6	2,83

QUADRO 6 — *Totais*

Grupos etários	Exames	Esplenomegalia						Hepatomegalia										Hepatosplenomegalia								
		I		II		III		IV		Total		1		2		3		4		5		Total		Casos	Percentagem	
		Casos	Percentagem	Casos	Percentagem	Casos	Percentagem	Casos	Percentagem	Casos	Percentagem	Casos	Percentagem	Casos	Percentagem	Casos	Percentagem	Casos	Percentagem	Casos	Percentagem					
0-4	52	2	3,84	—	—	—	—	—	—	2	3,84	2	3,84	—	—	—	—	—	—	—	—	—	2	3,84	—	—
5-9	49	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
10-14	55	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
15-19	56	—	—	—	—	—	—	—	—	—	—	1	1,78	—	—	—	—	—	—	—	—	—	1	1,78	—	—
+ 20	346	—	—	—	—	—	—	—	—	—	—	5	1,45	—	—	—	—	—	—	—	—	—	6	1,73	—	—
Totais	558	2	0,34	—	—	—	—	—	—	2	0,34	8	1,43	—	—	—	—	—	—	—	—	—	9	1,60	—	—



Fig. 2 — Aspecto da aldeia do Guijá



Fig. 3 — Outro tipo de habitações na aldeia do Guijá



Fig. 4 — Aldeia do Guijá, habitações de colonos



Fig. 5 — Canal esquerdo do sistema de irrigação do vale do Limpopo. Foco importante de moluscos hospedeiros intermediários da bilharziose



Fig. 6 — Outro aspecto do canal esquerdo do sistema de irrigação do vale do Limpopo



Fig. 7 — Outro aspecto do canal esquerdo do sistema de irrigação do vale do Limpopo



Fig. 8 — Aspecto do canal direito do esquema de irrigação do vale do Limpopo.
Este canal, ainda não concluído, é também um foco de moluscos



Fig. 9 — Aspecto de uma machamba no vale do Limpopo, mostrando uma vala
de distribuição das águas onde não se encontraram moluscos

MINISTÉRIO DO ULTRAMAR

INSPECÇÃO-GERAL DO FOMENTO

PROVÍNCIA DE MOÇAMBIQUE

BRIGADA TÉCNICA DE FOMENTO E POVOAMENTO DO LIMPOPO

IRRIGAÇÃO DO VALE DO LIMPOPO

ESQUEMA GERAL

"Esta população em aumento que a agricultura não poderá alimentar ou se puder alimentar, não pode subsistir, está diante de nós em poucos anos como problema que demanda soluções, e não vejo outros senão a emigração colonial e mais intensa industrialização do País."

SAI 4742

SAI 4742



- LEGENDA
- | | | |
|----------------|-----------------|-------------------|
| CANAL | VALAS DE ENXUGO | DIQUE |
| CONDUTOR GERAL | PRINCIPAIS | ESTADOS AGRÍCOLAS |
| DIREITO | SECUNDÁRIAS | ESTRADAS |
| ESQUERDO | | CAMINHO DE FERRO |
| DISTRIBUIDORES | | DESCARGAS |
| DESAÍDEIAS | | |

RESUMO

O autor refere os resultados de um inquérito parasitológico levado a efeito entre 558 indivíduos de ambos os sexos, de idades entre os 6 meses e os 75 anos, de raça europeia, estabelecidos como colonos no esquema de irrigação levado a efeito no vale do Limpopo, na circunscrição do Guijá, distrito de Gaza, da província de Moçambique.

O inquérito baseou-se em análises de urinas e fezes e na palpação do baço e fígado a todos os indivíduos.

Tendo em consideração que se tratava de indivíduos com pouco mais de um ano de residência em Moçambique, tendo vindo de territórios onde não existe a bilharziose, a presença de 2,86 por cento de casos da forma vesical desta doença, acrescida do alto índice de prevalência existente entre os africanos da região, é um índice seguro de que estes colonos se encontram grandemente expostos à infecção.

A prevalência de parasitas intestinais é reduzida, sendo provável que as parasitoses despistadas já afectassem os examinados antes da sua chegada a Moçambique.

O autor considera estes inquéritos como fundamentais para uma boa referência aos trabalhos futuros a realizar na região, de que dá uma ideia sumária.

THE TREATMENT OF URINARY BILHARZIASIS WITH ANTIMONY DIMERCAPTOSUCCINATE (TWSb)

ERNST A. H. FRIEDHEIM

New York

A review of published and unpublished studies on the treatment of *S. haematobium* bilharziasis, performed in Egypt, Israel, Morocco and Southern Rhodesia, involving in all 323 clinical cases, indicates the following:

The lower limit of the effective total dose is 2.0 g, largely independent of body weight. The useful total dose for adults ranges from 2.0 g to 2.5 g.

The lower limit of the effective single dose is 0.2 g. The useful single dose for adults ranges from 0.3 g to 0.5 g.

The period within which the total dose must be administered to be effective ranges from 2 to 10 days (2, 5, 6).

The dosage scheme applied by Alves (1), to entire satisfaction as to therapeutic effect and tolerance, i.e., 0.4 g per day for 4 consecutive days, may be taken as a base for routine treatments. It is suggested that the reproducibility of the therapeutic effect may be enhanced by increasing the number of 0.4 g doses to at least 5.

Intramuscular and intravenous applications are substantially equivalent as to effectiveness and tolerance.

The elimination of viable ova ceased in 95 percent of 72 cases 3 weeks, in 95 percent of 123 cases 4 weeks after the onset of a 3 to 10 day treatment with 1.0 g to 2.5 g (5, 6), and in 100 percent of 25 cases 3 to 6 weeks after 4 daily injections of 0.4 g (1).

Ova eliminated after subtherapeutic doses may be living, but incapable of hatching (5, 6).

Relapse-free presumptive cures are reported in all of 22 patients 6 months after a 10 day intramuscular treatment with 2.0 g (3); in

all of 49 patients 9 months after an intensive 2 day intramuscular treatment with 1.7 g to 2.4 g (4); in all of 19 patients 3 to 4 months after a 4 to 8 day intravenous and intramuscular treatment with 1.7 g to 3.1 g (2); in all of 26 patients not exposed to re-infection 12 months after a 4 to 7 day intravenous treatment with 1.8 g to 2.3 g (6).

In a group of 125 patients treated intramuscularly and intravenously with 1.0 g to 2.5 g for 4 to 7 days, the relapse rate was 2 percent in 98 patients after 1 to 2 months; 8 percent in 62 patients after 2 to 3 months; 1 percent in 54 patients after 3 to 4 months; 2 percent in 60 patients after 4 to 17 months (6).

Alves (1) reports absence of objective side effects in the ambulant treatment of 25 African schoolboys with 4 daily intravenous injections of 0.4 g. The other authors observed transient side effects, mostly gastro-intestinal, varying as to incidence and intensity in connection with the general condition of the patients. Cardiac side effects are limited to transitory ECG changes involving the amplitude and the direction of the T wave (3, 4). Padeh (4) concludes, in agreement with Salem *et al.* (5, 6), Goldstein *et al.* (3), Friedheim (2), «that TWSb does have toxic effects, especially when administered in more concentrated dosages to children or patients in poor general condition. However, in comparison with tartar emetic, these effects are much milder and of no practical significance because of the short course of the therapy and their rapid subsidence after the drug is stopped».

In conclusion, the 323 clinical observations reviewed here indicate that antimony dimercaptosuccinate (TWSb) administered for 2 to 10 days allows for a relatively well tolerated therapy of urinary bilharziasis, yielding better than 90 percent presumptive cure rates.

Of epidemiological significance are the findings indicating that 95 percent of the patients cease to eliminate viable ova within 3 weeks of the onset of the therapy.

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A NEW MOLLUSCICIDE: MOLLUSCICIDE BAYER 73

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In the fight against bilharziasis, molluscicides for the control of the vector snails have been gaining increasing importance. The products used heretofore — namely copper sulphate, pentachlorophenole (PCP) and, to a lesser extent, dinitro-*o*-cyclohexylphenole (DCHP) — have not, however, fully satisfied. In our search for new molluscicides we found new effective compounds, one of which — later termed Molluscicide Bayer 73 — appeared especially promising (Table 1).

Molluscicide Bayer 73 is chemically an aromatic acid amide, namely 2-hydroxy-5,2'-dichloro-4'-nitro-benzanilide (Fig. 1). The chemically pure compound is of grey colour, odourless and almost tasteless. As the compound is scarcely soluble in water, we searched for salts of better water-solubility and found the ethanolamine salt to appear especially suitable (Table 2). This salt has a yellowish colour.

TEST METHOD

Our test method is essentially the same as that employed in other institutes. Snails are immersed in beakers in the test concentration of a certain substance for 24 hours; subsequently they are washed in tap water and placed in fresh tap water for another 24 hours. Thereafter the number of killed snails is counted. A solution is rated as «effective» when all snails have been killed. If less than 50 percent

of the snails have been killed, the dose is regarded «ineffective». To a death rate of between 50 to <100 percent we refer as a «trace effect».

Especially interesting compounds are further tested in aquaria of up to 60 l capacity, in addition to the beaker test.

TABLE 1 — Toxicity of several molluscicides against *Australorbis glabratus*

Molluscicides	Concentration in ppm			
	3	1	0,3	0,1
Bayer 73.	+	+	+	—
PCP.	(+)	—	—	—
DCHP.	(+)	—	—	—
CuSO ₄	(+)	—	—	—

The following species of snails are available for these tests: *Australorbis glabratus*, *Limnaea palustris* and *Oncomelania hupensis*. *Australorbis glabratus* is chiefly used in these experiments.

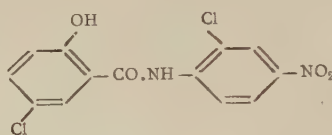


Fig. 1 — Chemical structure of Molluscicide Bayer 73

MOLLUSCICIDAL EFFECT

As may be seen from Table 1, Molluscicide Bayer 73 kills *Australorbis glabratus* quantitatively in a concentration of 0.3 ppm in tap water (pH 6.5-6.8) at temperatures of 22-24°C.

This result is obtained not only in the beaker test, but also in aquaria with luxuriant growth of aquatic plants even when contain-

ing a comparatively high deposit of mud and being well aired. Of PCP, DCHP and copper sulphate concentrations of at least 3 ppm are required to obtain the same effect in the beaker test.

Like the effect of other molluscicides, the effect of Bayer 73 varies with various species of snails. The minimum concentration required for the destruction of *Limnaea palustris* and *Oncomelania hupensis* is not 0.3 but 0.5 ppm; other snails, such as *Limnaea stagnalis*, are even more resistant.

With an exposure time of 24 hours, the kill rate (*Australorbis glabratus*) is largely independent of the temperature: the snails were killed by 100 percent in the temperature range from +4°C to +28°C.

TABLE 2 — Solubility of Molluscicide Bayer 73 and its ethanolammonium salt in milligrammes percent

	°C	Aqua dest.	Tap water
Bayer 73	+ 20	11	8
Bayer 73	+ 40	19	12
Bayer 73	+ 20	240	130
Bayer 73 salt	+ 40	280	185

With shorter exposure times differences in favour of the higher temperatures were seen.

Molluscicide Bayer 73, like pentachlorophenole, is effective against snail spawn and, therefore, superior to copper sulphate also in this respect.

The new molluscicide is better soluble in alkalinity than in acidity and, therefore, as with other molluscicides pH-dependent differences of effect are observed. Solubility and efficacy of the new compound are further dependent on the salt content of the water. In other respects Molluscicide Bayer 73 is, however, fairly stable. It is equally effective in well aired and nonaired aquaria. Microbial decomposition occurs only slowly, as could be concluded from orientating tests. Molluscicide Bayer 73 seems to have a higher stability to ultra-violet irradiation than PCP. Its adsorption to organic material is

relatively low; comparative studies with PCP showed no marked differences during 5 days of observation (Fig. 2).

The mechanism of action of the compound has not yet been explained satisfactorily. Experiments, using Warburg's apparatus, gave similar results as for PCP — namely, effective doses inhibit the respiration, lower doses accelerate it. However, the respiration is more effectively inhibited by Bayer 73 than by PCP, and of PCP

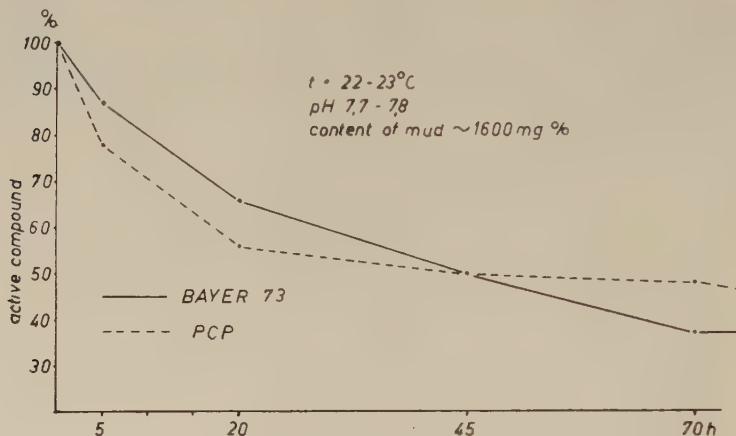


Fig. 2 — Adsorption of Molluscicide Bayer 73 and PCP by mud (mean values of 5 trials each)

about 20 times the Bayer 73-concentration is required for obtaining the same effect (Fig. 3).

Orientating field trials with Molluscicide Bayer 73 were carried out by Neuhaus¹, Halawani¹, Alves¹ and Gillet and Bruaux. The preliminary results are promising and justify further comparative investigations.

TOXICOLOGICAL STUDIES

Toxicological tests with Molluscicide Bayer 73 by Hecht¹ showed that the product has very little toxicity on single and repeated oral administration. Mice tolerated single oral doses of 2 g/kg, rats of

¹ Personal communication.

5 g/kg. In chronic feeding tests rats received a total of 30 daily doses of 0.250 g/kg on consecutive days except Sundays. The test animals showed no divergencies from the controls. The blood picture also remained normal. A young cow of 230 kg body-weight, being given 2.3 g orally a day for 93 days (total 223.9 g), remained healthy throughout the test; no retardation of growth or weight increase was discernible.

Also on subcutaneous administration the product proved to be little toxic. Mice tolerated 6 daily doses of 1 g/kg, but died when the dosage was raised to 2.5 g/kg. On intraperitoneal administration the substance is more toxic; the LD_{50} for rats was 0.1 g/kg.

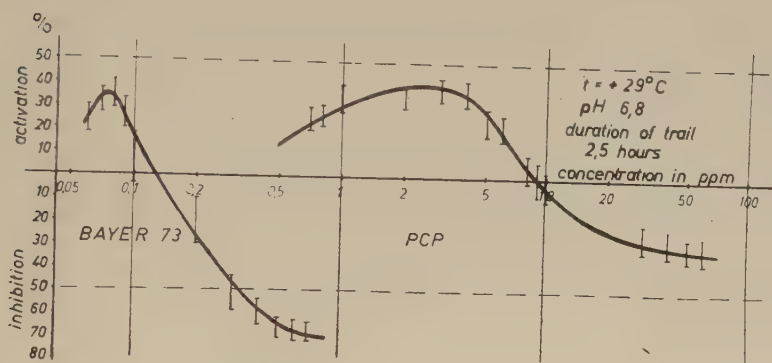


Fig. 3 — Consumption of oxygen by snails under the action of Molluscicide Bayer 73 and PCP

The compound was tested for its local irritative effects on rabbits and man. When the moistened powder was allowed to act on the skin for 24 hours, only one of 7 persons had a mild, rapidly vanishing irritation. Dust-chamber experiments showed that repeated exposure caused no mucosa irritation of the test animals. Bayer 73 and its ethanolamine salt have so little toxicity for warm-blooded organisms that no harm to humans or livestock is to be expected even if the treated water is occasionally used for drinking purposes.

The toxicity for fish was tested on *Lebistes* (Guppy) and *Xiphophorus* (swordfish). The LD_{50} amounts to about 1 ppm. A comparative fish-toxicity test showed Bayer 73 to be superior to PCP.

While PCP is 3 times as toxic for fishes as it is for snails, exactly the opposite ratio is true for Bayer 73 (Table 3).

Tests for phytotoxicity showed that for the plants tested — namely grass, mustard, oat-plants and rice — a damage is only to

TABLE 3 — *Toxicity of Molluscicide Bayer 73 and PCP against Australorbis (S) and Lebistes (F)*

	3 ppm		1 ppm		0,3 ppm	
	S	F	S	F	S	F
Bayer 73	+	+	+	(+)	+	—
PCP	(+)	+	—	+	—	—

be expected with concentrations of 100 ppm or more. These laboratory tests should be verified by field trials.

USE

For laboratory tests and field trials Molluscicide Bayer 73 is at present available as a 70 percent wettable powder, which meets the W.H.O. requirements for a DDT wettable powder.

SUMMARY

Summing up, it can be said that Molluscicide Bayer 73 is a new product which may be suitable for the control of snails. According to laboratory investigations, it is by 10 times superior to copper sulphate, pentachlorophenole and dinitro-o-cyclohexylphenole. The compound does not irritate either the skin or mucous membranes. It has remarkably little toxicity for warm-blooded organisms. Its fish-toxicity in relation to snail-toxicity is lower than that of PCP. According to the results available, plants are not affected by concentrations considerably exceeding those required for the destruction of snails. The suitability of Molluscicide Bayer 73 for the control of snails in the fight against bilharziasis should receive further investigation by field trials in various waters.

SCHISTOSOMIASIS IN THE TIGRIS-EUPHRATES VALLEY, WITH SPECIAL REFERENCE TO ITS ECONOMIC CONSEQUENCES

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INTRODUCTION

This study is concerned with the incidence and geographical distribution of human schistosomiasis, due to *Schistosoma haematobium*, in the valley of the Tigris and Euphrates rivers, based upon the results of a series of surveys carried out during the decade 1948-1957.

The work was sponsored principally by the Government of Iraq, but facilities were also granted by the Iranian Government and the Anglo-Iranian Oil Company.

THE INCIDENCE AND GEOGRAPHICAL DISTRIBUTION OF HUMAN SCHISTOSOMIASIS

The principal area of endemic *Schistosoma haematobium* in the valley of the twin rivers lies within the territory of Iraq, but two areas of endemicity are situated on tributaries of the Upper Euphrates in the regions of Koubour El Bid and Tel-Abiad, in northern Syria; while in the southern part the principal focus of endemicity extends over the border of Iraq into the Khuzistan Province of southwest Iran.

The human disease is entirely due to *Schistosoma haematobium*. No *Schistosoma mansoni* infections have been found in these areas.

The Iranian focus is continuous with the Iraqi focus; but the Syrian foci are isolated therefrom.

Evidence exists that the disease was endemic in this part of the world as far back as Babylonian times; and opinion differs as to whether the infection extended down the valley from the Syrian foci or northwards from the southern marshes.

The situation in the Syrian foci has been investigated by Pipkin and Rizk (1949), by Abdel Azim and Gismann (1956), and by Helmy (1952). The Iranian focus has been reported upon by Watson (1953), and by Ansari *et al.* (1953).

The major focus comprises the central and southern Provinces of Iraq. The disease does not now extend into the four northern Provinces, the few isolated foci formerly found there having been eradicated by mass treatment and anti-snail measures.

The infection is not found further south than Basrah, owing to the periodical incursion into the Shatt-el-Arab estuary of high salinity water from the Persian Gulf, which destroys the young vector snails.

The incidence figures for the central and southern Provinces of Iraq are given in the accompanying table. It will be noticed that those southern Provinces — Basrah, Muntafik and Amarah — which enclose the great marshes, show the highest overall prevalence of schistosomiasis. The average infection rate falls in the central Provinces of Diwaniyah, Kut, Hillah, Kerbela, Dulaim and Diyala. Prevalence also decreases as one proceeds northwards, with the remarkable exception of Baghdad Province, where a high endemicity is again found, in part owing to an influx of infected persons from the south and in part owing to the creation of favourable habitats for the vector snails by new irrigation works. It must be realised, however, that the incidence of schistosomiasis is very patchy, depending upon local conditions for snail-man contact, and for the breeding of the vector snails. For these reasons small zones of hyperendemicity are found — sometimes confined to a single village — scattered throughout the central and southern Provinces in an irregular manner.

EPIDEMIOLOGICAL NOTES

The infection rate for the central and southern Provinces as a whole, regardless of age, sex or place of origin, is just over 25 percent;

from which it may be deduced that upwards of one million persons are infected. Statistical analysis of the survey results showed remarkably little difference between different population groups: school children (24 percent), ambulatory adults (27 percent), hospital patients (28 percent), jail inmates (19 percent); the last-named group were, of course, free from the risk of reinfection. Surprisingly, urban and rural areas showed no significant difference over the country as a whole, perhaps owing to the fact that irrigation channels, through which infection may be acquired, penetrate even the densest urban aggregations. Breakdown showed, however, that in the Provinces of Kut, Amarah, Kerbela and Diyala, country-folk were more frequently infected than town-folk; but this was balanced by higher infection rates of townspeople in Basrah and Dulaim Provinces.

Further analysis showed that schistosomiasis in this area is a social rather than an occupational disease, associated with locality rather than calling; and that incidence falls as financial status rises. High incidence is associated with low income and poor housing conditions in close proximity to infected waters. Low incidence, on the other hand, is associated with better financial status and therefore with better housing conditions, with availability of pure, non-infected water for household use, and often also with regular and compulsory medical attention (Watson, 1953*b*).

Incidence varies little with age among the poorer classes of the population, when the source of infection is close at hand; but when the source of infection is distant, a higher incidence is found among the younger, more active, members of the population (Watson, 1953*a*).

High incidence is associated with cultivation of dates, rice, fruit and vegetables, which necessitate frequent contact with irrigation water at all seasons, rather than with cultivation of wheat and barley, crops which require special attention mainly at sowing and harvest.

No significant difference in incidence was found between areas with lift irrigation by pumps and areas with flow irrigation by gravity canals. Irrigation channels which contain water permanently and are subject to periods of stagnation are more often infested with the vector snails than those which dry out frequently or contain rapidly flowing water. Culverts and drains provide particularly favourable habitats for the snails (Watson, 1951).

EXTENSION OF CONTROL

The extensive schemes for the expansion of the irrigated areas, which are currently being carried out, will provide abundant new favourable habitats for the molluscan host, a situation which is being taken into account by the Iraqi Public Health authorities in their campaign to control human schistosomiasis.

Investigations of the bionomics of the snails are being intensively pursued with the object of finding means of environmental control. Weed-clearance and the application of molluscicides are extensively used.

The number of fixed and mobile clinics is being increased in order to permit detection and treatment of all infected persons.

The improvement of economic conditions and social standards, which is likely to take place in the area in the immediate future, will have a marked effect in reducing the incidence of human schistosomiasis.

SCHISTOSOME DERMATITIS

This cutaneous infection is widespread in southern Iraq and occurs sporadically elsewhere. It is not yet certain whether it is due to a distinct species of schistosome normally parasitic in a definitive host other than man, or whether it is a form of immunity reaction due to the cercariae of *Schistosoma haematobium*. The former hypothesis is more likely to be correct, in view of the very large number of water birds which frequent the southern marshes (Watson & Najim, 1956).

ANIMAL SCHISTOSOMIASIS

Cattle, sheep and goats in the lower part of the Tigris and Euphrates valley are frequently infected with *Schistosoma bovis* and *Ornithobilharzia turkestanicum*, of which the intermediate hosts are *Bulinus truncatus* and *Lymnaea lagotis* respectively.

Since the pre-war work of MacHattie (1936) and of MacHattie and Chadwick (1932) little progress has been made in the study of these infections.

Infection-rate with Schistosoma haematobium in Iraq

School	Population			Hospital			Gaol			Province	Urban			Rural			Total			
	Number of persons examined (by single urine examination)	Number of persons examined (by single urine examination)	Number found positive	Percentage of infected persons	Number of persons examined (by single urine examination)	Number found positive	Percentage of infected persons	Number of persons examined (by single urine examination)	Number found positive		Number of persons examined (by single urine examination)	Number found positive	Percentage of infected persons	Number of persons examined (by single urine examination)	Number found positive	Percentage of infected persons	Number of persons examined (by single urine examination)	Number found positive	Percentage of infected persons	
4,968	1,495	2,154	632	29	248	56	23	—	—	Basrah . . .	4,526	1,431	32	2,844	752	26	7,370	2,188	30	H
1,198	541	1,043	391	37	964	318	33	75	23	Muntafik . . .	1,638	637	39	1,642	636	39	3,280	1,273	39	H
8,106	749	831	289	35	392	139	35	—	—	Diwaniyah . .	836	243	29	3,493	934	27	4,329	1,177	27	H
1,548	270	950	125	13	50	14	28	—	—	Hillah . . .	548	20	4	2,000	389	19	2,548	409	16	M
1,219	275	810	241	30	432	130	30	100	14	Kut . . .	1,351	300	22	1,210	360	30	2,561	660	26	M
1,550	584	1,052	302	29	145	50	34	—	—	Amarah . . .	1,606	386	24	1,141	550	48	2,747	936	34	H
1,419	439	398	160	40	692	185	27	—	—	Baghdad . . .	1,219	367	30	1,290	417	32	2,509	784	31	H
1,792	272	40	12	30	60	10	17	—	—	Kerbela . . .	1,530	205	13	362	89	25	1,892	294	16	M
2,927	463	945	244	26	100	31	31	197	35	Romadi . . .	1,744	380	22	2,425	393	16	4,169	773	19	M
1,806	197	253	57	23	250	16	6	—	—	Diyala . . .	565	43	8	1,744	227	13	2,309	270	12	M
170	9	399	15	4	—	—	—	—	—	Mosul . . .	—	—	—	569	24	4	569	24	4	
68	0	269	8	3	68	1	1	—	—	Erbil . . .	405	9	2	—	—	—	405	9	2	
—	—	110	3	3	—	—	—	—	—	Kirkuk . . .	110	8	—	—	—	—	110	—	—	
21,771	5,294	9,254	2,479	27	3,401	950	28	372	72		16,078	4,024	25	18,720	4,771	25	34,798	8,795	25	

ECONOMIC IMPORTANCE

The importance of schistosomiasis to the community is sometimes underestimated.

It predominantly affects the poorer members of the population, but, for this very reason, its effect on the community is the greater, since the majority of the workers — upon whose efforts the prosperity of a nation ultimately depends — belong to this group.

Unfortunately, existing methods of investigation do not yet permit an accurate assessment, even of the effect on the individual, much less the correct determination of the medical, economic and social effects of this infection in a community. In particular, exact quantitative information is not yet obtainable in respect of:

- a) Prevalence;
- b) Worm burden;
- c) Pathogenicity;
- d) Immunity;
- e) Morbidity and mortality.

However, despite these difficulties, certain broad conclusions may be drawn from established facts and observations. Thus, evidence exists that even in relatively light infections some degree of anaemia and asthenia, with nervous and mental disturbance, may develop — leading eventually to debility, weakness and incapacity for sustained physical and mental effort; while, in addition, in children there may be retardation of physical and mental growth.

In heavy infections of long standing, where tissue damage has been severe, the injuries inflicted by the parasites may be crippling.

It is evident that individual suffering and loss of working capacity must result; but this may not be immediately evident since infected individuals unconsciously adapt their output of effort to their lowered strength, and it is only when they are subjected to heavy labour that their physiological balance breaks down and subjective as well as objective symptoms of schistosomiasis suddenly appear.

The world total of persons infected with schistosomiasis haematobia in Africa and the Middle East has been estimated by Stoll

(1947) at 39.2 millions, while statistics published in 1945 by W.H.O. put the figure higher at 50 millions. Clearly this disease is a matter of major concern. Moreover, unlike malaria, which is decreasing in importance under the combined effects of improved methods of treatment, effective control of the insect vectors and large-scale, well coordinated eradication campaigns, schistosomiasis is showing both increasing incidence and spreading geographical range, owing to the fact that comparably satisfactory methods of treatment and intermediate host control are not yet available, while planned eradication measures on an adequate scale are only just beginning to be attempted.

Some interesting light on the possible extent of the losses suffered by infected communities was thrown by Ansari (1955), who, on the basis that a person infected with vesical schistosomiasis would lose from 4 to 20 percent of his energy and working power, calculated that the annual reduction in agricultural output in Egypt alone, due to this cause, would amount to between £E 65,000,000 and £E 100,000,000.

A similar calculation may be made concerning the annual loss in Iraq, based on the present survey. Accepting Ansari's estimate of individual loss of energy and working output as being approximately correct and taking into account:

- i) the difference in average incidence between Egypt and Iraq;
- ii) the differing population figures; and
- iii) the differing mean annual value of the agricultural labourers' output in the two countries;

it can be shown that Iraq suffers a lowering of agricultural output amounting to the sum of between ID. 6,500,000 and ID. 10,000,000, as a direct result of schistosomiasis haematobia; while, in addition, the infected individuals suffered loss in personal income due to absence from work.

Yet further income loss is sustained by the community and the infected individuals, in respect of time occupied by treatment. From 6 to 15 injections on different days are required and each injection involves a separate visit to hospital, clinic or dispensary, with a consequent loss of from 2 hours up to a complete working day on each occasion, in addition to community expenditure on treatment.

Control work involves still greater communal expenditure, amounting in Egypt, for example, to over £E 1,000,000 a year.

These comments do not take into account the very serious economic loss involved in schistosome infections of domestic livestock; nor do they make any attempt to assess the loss consequent upon schistosome dermatitis, which may involve individual absences from work of up to 8 days.

CONCLUSION

The foregoing comments have attempted to associate incidence with economic consequences of endemic schistosomiasis haematobia in one of the important endemic foci of this disease.

If all three forms of schistosomiasis be considered in comparable fashion, with respect to all parts of the world where they are endemic, it will be seen that this disease is, perhaps, the major tropical health problem of our time.

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DRUG EVALUATION IN EXPERIMENTAL *SCHISTOSOMA* *MANSONI* INFECTIONS OF RHESUS MONKEYS¹

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The albino mouse has been a valuable animal in the screening of compounds in the quest for a better oral therapeutic agent for human schistosomiasis. It is desirable, however, to evaluate active compounds secondarily, especially in animals more akin to man, before extending efforts into pharmacological evaluation and eventual possible trial in humans. In the case of *Schistosoma mansoni*, rhesus monkeys are suitable for this purpose.

Experimental *S. mansoni* infections in monkeys have been reported by various workers. Whether the concern of investigation be pathology, chemotherapy, immunity, or basic biology, the evaluation of such studies should be based on as near a standard infection as possible, that is to say, a fairly accurately known population of worms. If such is lacking, it sometimes become difficult for others to extend or re-study on a comparative basis the various aspects of schistosomiasis. For example, if in an infected animal one assumes a susceptibility differential with respect to a given number of worms exposed to a given amount of drug, or any other explanation as to the factors involved

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in obtaining a partial effect or kill, it is not difficult to see that evaluations based on varied or unknown numbers of worms can be quite different.

In view of the fact that the intraperitoneal infection of mice results in about 50 percent of the worms remaining submature in the peritoneal cavity, and because it has been found in this laboratory (unpublished) that immature peritoneal worms are found in mice infected subcutaneously, and since (expecting possibly antimonials, Schubert, 1948) no compounds are active against immature worms, these two methods of infection would seem less desirable in the evaluation of drug action against mature worms. Until it has been demonstrated that the intraperitoneal infection of monkeys does not result in the development of worms in the peritoneal cavity, the percutaneous route certainly should be used and the number of cercariae stated as accurately as possible. The infection of monkeys by placing unspecified numbers of cercariae in drinking water, the buccal cavity, floor of cage, or on the skin should be avoided for obvious reasons of variability and error. Successive exposures of the animal to cercariae, either closely or widely spaced, because of immunological influences complicate the estimation of worm burden and conceivably result in worms of varying maturity unless a few weeks have elapsed between each exposure. Even so, in such cases the age of the mature worms would be different.

It has been found in this laboratory that the percutaneous exposure of rhesus monkeys (weight range, 2.5-4 kg) to 1,000 cercariae for 45 minutes, accomplished under intravenous anesthesia to the animal and attaching with adhesive tape to the shaved lower abdomen a 3 cm stender dish containing the cercariae, results in the maturity of about 250 worms. The cercariae are taken from a pooled sample of 15 to 20 snails, in this case *Australorbis glabratus* known to be infected with both sexes of cercariae. By taking a dozen 0.1 ml aliquots of the cercarial suspension, which is continuously agitated by a magnetic stirrer, the mean number is adjusted until there are 20 cercariae per 0.1 ml. 5 ml of the sample are then placed into the stender dish. A cercarial dermatitis outlining the dish follows in a few hours and persists at the exposure site for at least 24 hours. Over 80 monkeys have been used in the secondary evaluation of compounds

active against a Puerto Rican strain of *S. mansoni* and all became infected.

Between the 7th and 8th week there is, with very few exceptions, an average weight loss of 8 percent in the monkeys, accompanied by amorphous (54 percent) or mucoid diarrhetic (34 percent) stools, with or without blood flecks. In following two control animals through 18 months, there was no additional diarrhea except on a single occasion between the 10th and 12th week. The stool texture is followed during treatment and post-treatment, but is complicated by the fact that many compounds seem to induce fluidity of the feces.

Numbers of eggs in the feces, as determined by a modified Telemann acid-ether technique, showed that ova per unit displacement vary in dividual samples passed within a 24-hours period and in replicate counts made on a single unit displacement, the former to a high degree of statistical significance. In a few comparative tests on the same stool sample the AMS III concentration method did not appear to be more sensitive. Feces negative by acid-ether concentration are subjected to either sedimentation or centrifugation hatching techniques on a 10 g sample. Either method works well.

Eggs first appear in the feces 37 to 46 days (average = 42) after infection, and in 81 percent of the animals (based on 2-3 different samples the week preceeding treatment) and per milliliter of displaced feces were 150-450; in 19 percent of the animals, 550-1,150. Eggs in single stool specimens taken on the 1st day of treatment numbered 200-1,800 per unit displacement. In 3 control animals peak numbers occurred between 8 through 11 weeks, followed by a rather sharp decline which extended into relatively low sustained numbers until about the 7th month. After this time 1 animal showed sporadic egg findings until 20 months, while the other 2 controls passed eggs consistently after 9 and 20 months respectively.

A single monkey, sacrificed and examined at 8 weeks, showed a total of 294 worms, of which 16 percent were in the portal, 79 percent in the superior and inferior venous networks (twice as many in the inferior as in the superior); and 5 percent in the liver. The latter organ, in addition to its worms, had numerous egg lesions of the pseudotubercule type along with bilharzia pigment. There were extensive egg lesions along the descending and transverse colon, considerably fewer on the ascending branch, but not grossly visible along the entire

small intestine. Since eggs are recovered from the feces as early as 5 weeks after infection, it would seem that an 8-weeks-old infection in monkeys is mature, and in view of the time apparently required for the eggs to leave the vascular system and void themselves in the feces, maturity must be reached considerably earlier. Additional data from monkeys dying early in therapy indicate a burden of about 250 worms.

Drugs, either as suspensions or solutions in 30 ml and based on weekly body weights, are given at 6-8 weeks after infection, depending on whether 2 or 3 positive egg counts can be demonstrated by acid-ether. Treatment is by gavage twice daily, 5 days a week for 1 to 4 weeks. During the entire treatment and post-treatment periods, the feces are examined twice weekly by acid-ether and by hatching, if the former is negative. If the animal shows 6 consecutive negativities toward the end of the holding period (at least 5 weeks), it is sacrificed and perfused. An extended holding period is an important feature of the evaluation because it has been found that in many treatments eggs could not be demonstrated by either concentration or hatching only to recrudesce again to moderate or low counts. It is possible to use the same animal for two or three courses of treatment as long as living eggs can be demonstrated.

After the animal is sacrificed it is perfused with 3 l of sodium-citrate-saline by cannulating the left ventricle and collecting from the right. The cleared veins are inspected with a 3X Fisher Magnifier-Illuminator. The liver is passed through a meat grinder followed by pressing small amounts between 6"×8" glass plates and then examined with a dissecting microscope. The perfusate is lysed and inspected. By this method living worms are almost always washed into the liver, and have never been found in the perfusate.

With active compounds there is tissue reaction around dead worms in the liver and a reduction of egg lesions both in the liver and bowel. There is every indication that with active drugs the worms drift into the liver, as is true in mice, and that killing takes place in that organ. Cured cases, examined 5 weeks after the end of treatment, do not show eggs in the gut or liver, usually no pigment in the liver, or no dead worms in the liver or veins. This indicates that in monkeys the elimination of ensheathed dead worms and eggs requires no longer than 8 weeks.

Thus, the main points of evaluation are: (1) The absence or reduction of eggs in the feces, bowel, and liver; and (2) The absence or — if present — the condition of worms primarily in the liver of perfused animals. The latter factor can indicate the rapidity of drug action.

Therapeutic data with Miracil D using 9 monkeys were as follows: a single dose of 100 mg/kg ineffective, and in doses of 25, 50, 100 and 200 mg/kg twice daily for 5, 10, or 20 days were moderately effective. In some of these treatments there was a pronounced *transient* loss of eggs in the feces, evidenced by the inability to demonstrate eggs by either concentrative or hatching technique. Without the extended holding period one could get an inaccurate idea of complete effect, which would result in the unnecessary sacrifice of the animal. These results in which no cure was obtained, even at maximum tolerated levels, show less than expected activity when compared with the results of other workers. To what degree the difference stems from schistosome strain, mammal, or near-standard infection and reference of evaluation, is difficult to say. In any case and in summary, the discussed procedure and methods permit the appraisal of either partial therapeutic effect, and more confidently, of complete cure.

ACÇÃO DO RICINOLEATO DE COBRE E DE OUTROS PLANORBICIDAS SOBRE AS CERCÁRIAS DO *SCHIS-* *TOSOMA MANSONI*¹

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e

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Uma solução planorbicida ideal deverá possuir, além da acção directa imediata, em elevada diluição, sobre esses moluscos, também acção retardada, conservando-se por longo tempo activa nas águas tratadas.

Tendo em vista encontrar um produto que satisfizesse essas condições, propusemos, em trabalho anterior (1), o emprego como moluscocida do ricinoleato de cobre, um sal insolúvel na água, mas facilmente emulsificável, capaz de manter essa emulsão por muito tempo, aderindo, além disso, à vegetação utilizada pelos moluscos como alimentação e para deposição de seus ovos na superfície das folhas.

No presente trabalho procuramos verificar, comparativamente, a acção do ricinoleato de cobre, como também ricinoleato de sódio, sulfato de cobre, pentaclorofenato de sódio e verde-paris, sobre as cercárias do *S. mansoni*. Os resultados obtidos na realização dessas experiências pouco significariam se empregássemos simplesmente diluições na relação peso para volume. Procurámos então fazer as diluições das soluções a serem experimentadas, de modo a estabelecer uma relação ou um elo comum a todas.

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O sulfato de cobre cristalizado contém em sua molécula cerca de 36 por cento de água de cristalização, que nada contribui para sua actividade. Só podemos comparar essa actividade com a exercida pelo ricinoleato de cobre se procurarmos um denominador comum existente nas duas moléculas, que no caso é o óxido de cobre, a que podemos reduzir ambas as substâncias, por combustão em cadinho de porcelana.

Contudo, no decorrer de nossos trabalhos verificámos que também o radical ricinoleico possui acentuada actividade contra as cercárias do *S. mansoni*. Assim, para esclarecermos a acção do ricinoleato de cobre sobre as cercárias, pareceu-nos interessante investigarmos a contribuição trazida para essa actividade pelo radical ricinoleico existente no ricinoleato de cobre, empregando na dosagem quantidade de ricinoleato de sódio equivalente à existente nas diluições de ricinoleato de cobre ensaiadas.

Assim, as soluções mencionadas no quadro explicativo que apresentamos em seguida se referem às seguintes relações:

Ricinoleato de cobre: dosagem em CuO .

Ricinoleato de sódio: diluições contendo quantidades equivalentes às existentes no ricinoleato de cobre.

Sulfato de cobre: dosagem em CuO .

Pentaclorofenato de sódio: nas diluições de peso para volume.

Do mesmo modo, consideramos como termo de comparação, neste quadro, os tempos de 4 e 14 minutos, respectivamente, para a acção das soluções experimentadas sobre a movimentação e mortalidade das cercárias.

Ao confrontarmos no quadro seguinte as diluições necessárias para produzir a imobilização das cercárias do *S. mansoni* no período de 4 minutos teremos a seguinte sequência de actividades: o *ricinoleato de sódio* (parte equivalente à existente no ricinoleato de cobre) é mais activo do que o *ricinoleato de cobre*, este, de actividade igual à do *pentaclorofenato de sódio*, sendo menos activo o *sulfato de cobre* que todos esses compostos.

Em relação às diluições necessárias para produzir a morte das cercárias em 14 minutos, teremos a seguinte sequência de actividades: o *ricinoleato de sódio* (a parte equivalente) é mais activo do que

o *ricinoleato de cobre*, que, por sua vez, é mais activo do que o *pentaclorofenato de sódio*, que é mais activo do que o *sulfato de cobre*.

Título das diluições (em milhões)	2,5	5,0	10,0	15,0	20,0	24,0
PERDA DOS MOVIMENTOS:						
1. <i>Ricinoleato de sódio</i> Números absolutos, representando diluições de peso para volume.	18'	< 1,30 h				
2. <i>Ricinoleato de sódio</i> Números relativos, representando quantidades equivalentes do radical ricinoleico existente no ricinoleato de cobre.	Imediata	Imediata	1'	2'	3'	4'
3. <i>Ricinoleato de cobre</i> Diluições expressas em CuO existente na molécula.	1'	2'	3'	4'	5'	7'
4. <i>Sulfato de cobre</i> Diluições expressas em CuO.	9'	11'	13'	16'	18'	20'
5. <i>Pentaclorofenato de sódio</i> Números absolutos, representando diluições de peso para volume.	1'	2'	3'	4'	5'	7'
MORTALIDADE:						
1. <i>Ricinoleato de sódio</i>	0	0	0	0	0	0
2. <i>Ricinoleato de sódio</i>	2'	3'	4'	5'	8'	16'
3. <i>Ricinoleato de cobre</i>	6'	8'	10'	14'	24'	32'
4. <i>Sulfato de cobre</i>	14'	16'	22'	28'	31'	33'
5. <i>Pentaclorofenato de sódio</i>	6'	9'	14'	16'	24'	30'

Verificamos, assim, que a presença do radical ricinoleico traz uma apreciável melhoria à acção do ionte cobre.

As experiências foram realizadas de forma que cada solução apresentasse, depois da adição do liquido contendo as cercárias, uma

determinada concentração, sendo imediatamente examinados estes parasitas ao microscópio entomológico, quanto à sua motilidade através do líquido (poder infestante) e ao tempo que decorria até que se apresentassem inertes e mortos no fundo do vidro de relógio em que estavam contidos. As cercárias testemunhas, não tratadas, mantiveram-se vivas e activas e as provas biológicas de infestação experimental em mamíferos demonstraram a sua plena vitalidade.

A actividade maior do ricinoleato de sódio pode ser interpretada como devida à acção detergente desse sabão, privando talvez as cercárias da camada protectora que as impermeabiliza à penetração da água.

O ricinoleato de cobre, sendo insolúvel, age mais lentamente, não possuindo a acção detergente, mas mantendo por tempo prolongado a acção específica do cobre, que é activada pelo radical ricinoleico.

O sulfato de cobre, mais uma vez, evidenciou a acção específica deste metal.

Quanto ao verde-paris, sendo um composto insolúvel de cobre, deixou de figurar no presente quadro. Contudo, os resultados verificados com as diversas quantidades experimentadas demonstram que ele age como veneno de contacto, na dependência da dispersão em que se encontra na superfície do líquido.

Como conclusões desse trabalho, julgamos que:

1.º O ricinoleato de cobre tem acção acentuada sobre as cercárias do *S. mansoni*.

2.º Um excesso de ricinoleato de sódio nas emulsões de ricinoleato de cobre adiciona ao efeito moluscocida deste último sal a acção muito acentuada do sabão sódico contra as cercárias do *S. mansoni*, o que aumenta de muito o valor desse produto para as campanhas contra a esquistossomose.

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ESTUDO DO METABOLISMO EM MOLUSCOS DE ÁGUA
DOCE: IV — ABSORÇÃO DO CÁLCIO PELO *AUSTRA-
LORBIS GLABRATUS*, ESTUDADA COM O USO DO ^{45}Ca

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INTRODUÇÃO

Na sequência de investigações sobre o metabolismo em moluscos de água doce (*Gastropoda*, *Pulmonata*) realizadas por alguns de nós (1), apresentam-se os resultados obtidos no estudo da absorção do cálcio no *Australorbis glabratus*. Como é sabido, o papel daqueles gastrópodes é de grande importância em medicina tropical como hospedeiros de esquistossomas.

Neste trabalho utilizámos o *Australorbis glabratus*, porque, muito embora não sendo do ultramar português, é molusco de fácil manutenção e cultura em condições de laboratório.

Este *Australorbis* deve a sua importância ao facto de ser hospedeiro intermediário do *Schistosoma mansoni*, forma particularmente frequente na América do Sul, em especial no Brasil.

É bem conhecida a incapacidade temporária ou permanente, bem como o desgaste populacional, causados pelos esquistossomas, tanto neste país como nas nossas províncias ultramarinas, em especial Moçambique.

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O problema da absorção do cálcio e o seu metabolismo pelos gastrópodes é de estudo mais delicado do que o dos iões anteriormente estudados (1), em virtude do elevado teor em cálcio da concha do molusco, e ainda porque tal elemento não se encontra totalmente ausente da parte mole do corpo.

Bento de Magalhães Neto *et al.* (2) indicam para o *Australorbis glabratus* valores médios de percentagem de cálcio variando entre os seguintes limites: concha, 34,18 a 27,49 por cento; partes moles, 2,51 a 1,26 por cento.

O cálcio é, portanto, um elemento fundamental no metabolismo do molusco e a vida deste está permanentemente condicionada pelo teor naquele elemento do meio em que vive.

Romeiro e Aguiar (3) estudaram esta influência no caso do *Australorbis tenagophilus* e Harry *et al.* (4) apresentaram resultados relativos ao teor em cálcio das águas de Porto Rico, que têm condições de vida para o *Australorbis glabratus*. Assim, estes autores verificaram que aquele molusco vive em águas apresentando teores em cálcio que variam entre 17 e 129 partes por milhão (p. p. m.), sendo o intervalo óptimo para o seu *habitat* situado entre 17 e 70 p. p. m. Num estudo recente (5), Harry e Aldrich, procurando estabelecer relações entre os factores ecológicos e a repartição do *Australorbis glabratus*, verificaram que, ao contrário das variações de temperatura, de intensidade luminosa, de pH e de opacidade da água, um teor de carbonatos e bicarbonatos relativamente elevado — quando comparado com o teor de cloretos e sulfatos — pode explicar a ausência daquele molusco de certas zonas, como, por exemplo, dos ribeiros das regiões calcárias. Estes autores verificaram ainda que o *Australorbis glabratus* raramente se encontra em águas cujo teor de sólidos dissolvidos é inferior a 150 p. p. m. Contudo, tanto Harry e Aldrich, como nós, mantivemos em laboratório este molusco em água destilada.

O estudo realizado por nós baseia-se na utilização do cálcio radioactivo, ^{45}Ca .

Este isótopo é um emissor beta puro de baixa energia (0,25 Mev), sendo assim conveniente para a obtenção de autorradiografias em boas condições; esta energia, embora baixa, é, no entanto, suficiente para medição de actividade em contadores Geiger-Müller de janela.

O período de semidesintegração do ^{45}Ca — 164 dias — permite seguir um estudo deste género durante um intervalo de tempo suficientemente longo.

Por estas razões, o ^{45}Ca tem sido utilizado por diversos autores em problemas metabólicos de diferentes organismos.

Assim, entre outros, Rosenthal (6 e 7) estudou com aquele radioelemento o mecanismo de absorção do cálcio por alguns peixes de água doce. Note-se que o autor fez este estudo para determinar a possível contaminação dos seres vivendo em águas contaminadas pelos produtos de desintegração radioactiva que nelas são lançados.

No nosso caso interessa-nos, primordialmente, averiguar de que modo se faz a absorção do cálcio pelos moluscos e como ele se divide entre a concha e partes moles.

Em virtude disso, procurámos também averiguar a perda em cálcio pelo tubo digestivo e a quantidade deste elemento permanentemente retido no seu corpo.

Existe ainda controvérsia sobre o modo como os moluscos gastrópodes obtêm o cálcio de que têm necessidade, como se pode verificar, por exemplo, no trabalho de Fröemming (8) relativo a diversas espécies de gastrópodes.

MÉTODOS UTILIZADOS E RESULTADOS OBTIDOS

Em virtude de a parte experimental deste trabalho ter sido feita durante o Inverno, o qual, nas nossas latitudes, produz temperaturas sensivelmente mais baixas do que as que correspondem ao *habitat* natural do *Australorbis glabratus*, foi-nos necessário criar condições artificiais de luz e temperatura para que o ambiente laboratorial se assemelhasse, tanto quanto possível, ao ambiente natural. Para isso projectámos uma estufa convenientemente iluminada e aquecida por um sistema de *contrôle* automático de temperatura, que, a par de uma adequada circulação de ar, manteve aquela, em qualquer local da estufa, a $22 \pm 1^\circ\text{C}$.

Utilizámos 25 *Australorbis glabratus* adultos, da mesma geração, os quais foram colocados em copos cilíndricos, de vidro comum, de 250 ml de capacidade, 120 mm de altura e 54 mm de diâmetro. Cada copo continha 50 ml de solução activa preparada como adiante

se indica e apenas um exemplar daquele molusco; deste modo pudemos apreciar a absorção individual em cálcio dos gastrópodes em estudo.

Na preparação das soluções activas, nas quais mergulhámos os indivíduos em estudo, tivemos de ter em mente a sensibilidade dos métodos de medida radioactiva de que dispomos, considerando que queríamos observar a perda de actividade das soluções no decorrer do tempo, tomando amostras de líquido suficientemente pequenas para que a quantidade de material radioactivo extraído da solução fosse desprezável em face da quantidade total do radioelemento contido nela. De acordo com estas considerações, determinámos como suficiente uma actividade de $15 \mu\text{c}$ em cada copo.

A substância activa empregada foi o cloreto de cálcio ($\text{Cl}_2^{45}\text{Ca}$), fornecido pela Isotope Division, Harwell, Inglaterra. As soluções foram preparadas dissolvendo este sal em água destilada e empregando uma quantidade tal que, no início da experiência, o líquido de cada copo apresentasse a actividade acima indicada. Procurou-se também que o teor em cálcio das soluções estivesse dentro dos limites indicados por Harry *et al.* (4).

Durante todo o período experimental os moluscos foram mantidos sem alimentação para que o cálcio contido nesta não interferisse, para efeitos de absorção pelo molusco, com o cálcio radioactivo empregado. Com efeito, estes moluscos costumam ser alimentados em laboratório com um gel que contém alginato de cálcio e alface; mesmo empregando esta só, introduz-se uma razoável quantidade de cálcio estranho, pois aquele vegetal possui 24 mg deste elemento por 100 g de produto (9).

O estudo da absorção do cálcio pelos moluscos ensaiados foi conduzido utilizando dois modos distintos de observação: o primeiro foi orientado no sentido de se medir a perda, em cálcio, das soluções em que se encontravam os *Australorbis*; o segundo diz respeito à avaliação da quantidade de cálcio absorvida pelo corpo dos moluscos.

Paralelamente, observou-se qual a quantidade deste elemento perdida por catabolismo. Todas estas medidas foram feitas utilizando métodos radioactivos, sendo as actividades a seguir registadas corrigidas de tempo morto, de fundo do sistema de contagem e da deca-

dência física do ^{45}Ca . A contagem da actividade fez-se, em cada caso, num tempo tal que o erro de medição, de acordo com a estatística de Poisson, não ultrapassasse 3 por cento.

CÁLCIO PERDIDO PELAS SOLUÇÕES

A sua avaliação foi feita tomando amostras das soluções de $\text{Cl}_2^{45}\text{Ca}$, contidas nos copos, com o volume de 0,1 ml, medidas por meio de uma pipeta associada a um parafuso micrométrico.

Cada gota de líquido obtida foi colocada num porta-amostras de alumínio (do tipo usado no nosso sistema de contagens radioactivas) e uniformemente distribuída pela superfície deste com umas gotas de piridina; foi alcalinizada com amónia a 10 por cento e precipitada com carbonato de sódio a 5 por cento. Deste modo obtivemos amostras de cálcio radioactivo, sob a forma de carbonato, mecânica e quimicamente estáveis e todas nas mesmas condições geométricas para medida de actividade.

Preocupou-nos averiguar a existência de uma possível absorção própria da radiação beta emitida pelo cálcio 45 na massa das amostras obtidas segundo a técnica acima indicada. Com efeito, a radiação beta emitida pelo cálcio-45 é de energia suficientemente baixa para que seja de considerar tal absorção própria. Realizámos, portanto, um ensaio em que se mediu a actividade de uma amostra preparada como atrás se indicou, tendo-se obtido 7020 contagens por minuto (c. p. m.). Num segundo ensaio mediu-se a actividade de uma amostra em que se utilizou o dobro do volume da solução de cloreto de cálcio-45 utilizada no primeiro ensaio; obtiveram-se 13 951 c. p. m., que, considerando os erros estatísticos das medidas de actividade, está perfeitamente de acordo com o resultado obtido no primeiro ensaio. Não tivemos, portanto, de entrar em consideração com correcções devidas à absorção própria da radiação beta na massa das amostras, o que não aconteceria se a massa total fosse bastante superior.

Foi tomada uma amostra de cada copo no início do ensaio; obtiveram-se subsequentemente outras amostras até à data em que o respectivo molusco foi retirado.

As amostras foram preparadas como anteriormente se descreveu, tendo-se medido a sua actividade com um tubo Geiger-Müller, de janela, com a espessura de 4 mg/cm²*.

QUADRO 1 — *Actividades das amostras de liquido colhidas nos copos*

Copo n.º	Actividade do liquido (c. p. m. / 0,1 ml)				
	17 / III (dia zero)	22 / III (5.º dia)	25 / III (8.º dia)	28 / III (11.º dia)	8 / IV (22.º dia)
1	2 930	—	—	—	—
2	4 509	—	—	—	—
3	4 995	—	—	—	—
4	5 579	—	—	—	—
5	4 675	—	—	—	—
6	4 381	1 281	—	—	—
7	4 149	3 154	—	—	—
8	4 549	1 219	—	—	—
9	4 801	1 390	—	—	—
10	4 637	312	—	—	—
11	4 577	788	407	—	—
12	4 683	321	82	—	—
13	4 507	1 273	881	—	—
14	4 543	169	116	—	—
15	4 672	1 655	595	—	—
16	3 234	1 949	1 458	1 581	—
17	3 262	233	69	50	—
18	3 476	98	76	59	—
19	3 185	110	502	349	—
20	3 346	739	87	65	—
21	3 686	83	8	27	50
22	3 284	170	15	1	32
23	3 371	26	2	5	47
24	3 128	141	31	28	50
25	3 403	399	383	28	0

* Como se disse na introdução, a energia da radiação beta do ⁴⁵Ca, se bem que baixa, é suficiente para ser medida nos contadores Geiger-Müller do tipo indicado. Com efeito, utilizando a fórmula de Evans (10), pode-se avaliar em quase 60 por cento a radiação que, com a energia da emitida por aquele radioelemento, é transmitida através da janela do contador.

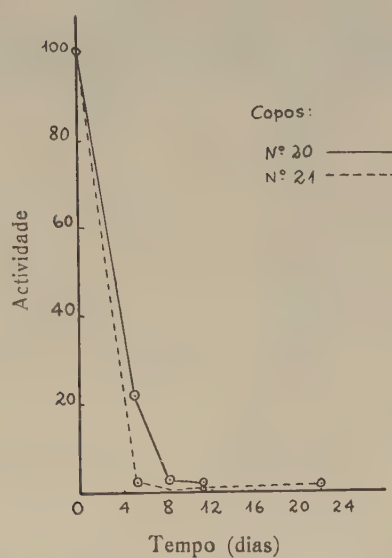
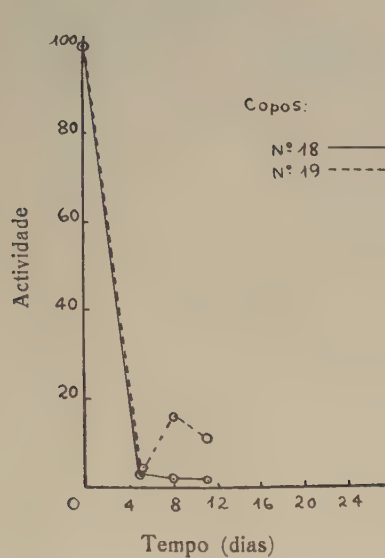
Os valores obtidos, nos diferentes copos, para as actividades das amostras dos líquidos contidos neles encontram-se indicados no quadro 1.

No quadro 2 encontram-se registados estes mesmos valores tomados como percentagens dos valores da actividade inicial (a correspondente ao dia zero).

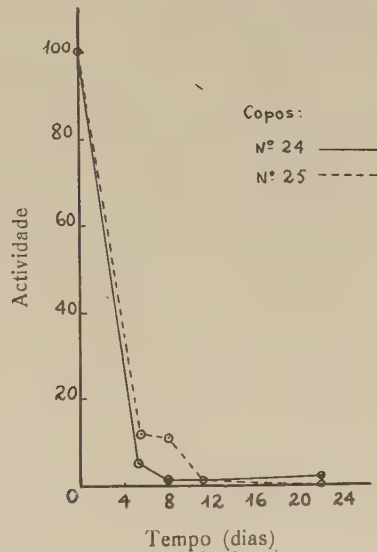
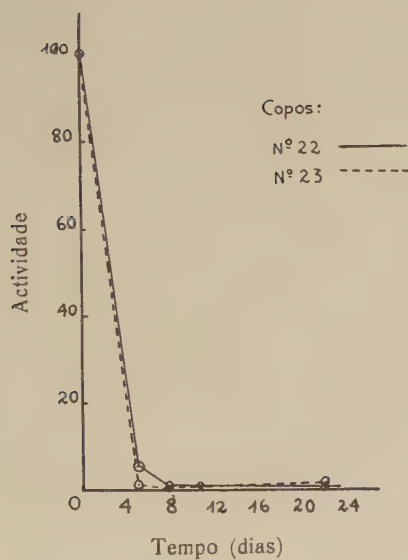
QUADRO 2 — *Valores do quadro 1 expressos em percentagens*

Copo n.º	Actividade do líquido em relação à actividade inicial			
	22 / III (5.º dia) %	25 / III (8.º dia) %	28 / III (11.º dia) %	8 / IV (22.º dia) %
6	29,2	—	—	—
7	75,2	—	—	—
8	26,8	—	—	—
9	28,9	—	—	—
10	6,72	—	—	—
11	17,2	8,89	—	—
12	6,85	1,75	—	—
13	28,2	19,5	—	—
14	3,72	2,55	—	—
15	35,4	12,7	—	—
16	60,3	41,1	48,8	—
17	7,14	2,11	1,53	—
18	2,81	2,18	1,66	—
19	3,45	15,7	10,95	—
20	22,0	2,60	1,94	—
21	2,25	0,217	0,732	1,35
22	5,17	0,456	0,030	0,974
23	0,771	0,059	0,148	1,39
24	4,57	0,991	0,895	1,59
25	11,7	11,2	0,823	0
Médias. . . .	18,92	8,13	6,75	1,06

Como exemplos de perda em cálcio dos líquidos contidos nos copos, construíram-se os gráficos 1, 2, 3 e 4. Eles correspondem aos oito últimos moluscos retirados das soluções activas. Com estes gráficos pode-se verificar que nos quatro primeiros dias os moluscos a



Gráficos 1 e 2 — Actividades dos líquidos contidos nos copos



Gráficos 3 e 4 — Actividades dos líquidos contidos nos copos

que dizem respeito absorveram quase todo o cálcio do meio em que viviam.

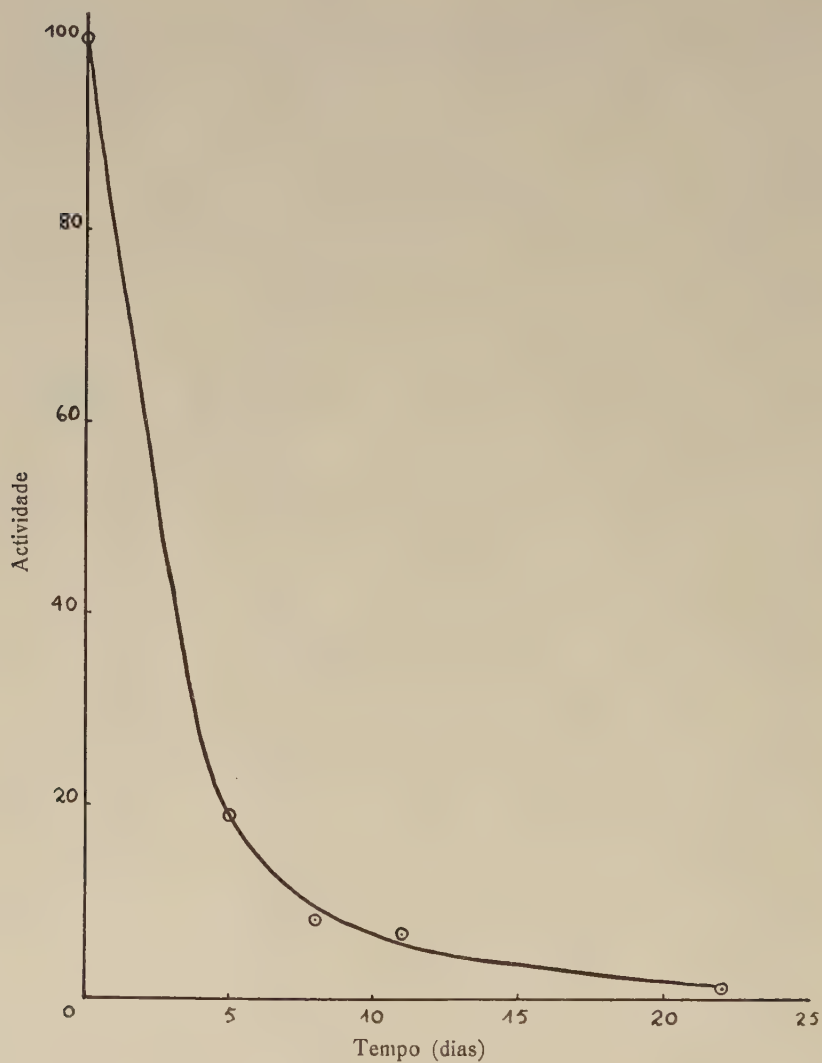


Gráfico 5 — Actividades médias dos líquidos contidos nos 25 copos

No final do quadro 2 indicam-se as médias das percentagens das actividades encontradas em todos os copos nos diferentes dias.

Com estes valores médios construiu-se o gráfico 5, o qual apresenta um comportamento análogo ao dos quatro primeiros gráficos, apesar das consideráveis diferenças na velocidade de absorção individual do cálcio.

CÁLCIO ABSORVIDO PELOS MOLUSCOS

Como atrás se disse, paralelamente à avaliação da perda de cálcio das soluções em que se encontravam os *Australorbis*, fez-se uma avaliação da actividade que o corpo destes ia absorvendo no decorrer do tempo.

Para isso dividiram-se os 25 moluscos de que dispúnhamos em cinco lotes iguais. Cada um desses lotes foi retirado do meio radio-activo nas datas indicadas no quadro 3.

Cada molusco foi então tratado do seguinte modo: separou-se a concha da parte mole, mergulhando o molusco em água a 70°C, durante 10 a 12 segundos, tempo necessário para que, puxando o pé com uma pinça, a parte mole se separe da concha sem resistência; com o fim de não danificar a concha, utilizámos uma pinça de pontas curvas.

A concha foi lavada, seca em estufa e pesada com o fim de converter os valores das actividades encontradas nos moluscos em actividades específicas, expressas em contagens por minuto por miligrama de concha (c. p. m./mg).

Posteriormente, a concha foi tratada com ácido clorídrico e a solução resultante alcalinizada com amónia e precipitada com carbonato de sódio. O precipitado foi separado da solução por meio de um sistema de filtração que utiliza discos de papel de filtro que podem ser perfeitamente ajustados aos porta-amostras anteriormente referidos, ficando, portanto, em condições de serem medidas as suas actividades. Estas encontram-se indicadas no quadro 3.

As partes moles foram calcinadas em cadinho fechado e tratadas duma maneira análoga à já indicada para as conchas. Únicamente se teve de juntar cloreto de cálcio estável em solução, como *carrier*, devido às diminutas quantidades de material precipitável pelo anião carbonado que se encontravam nas partes moles dos moluscos.

As actividades medidas, bem como as específicas, respeitantes às partes moles, encontram-se, tal como os valores correspondentes das conchas, indicadas no quadro 3.

QUADRO 3 — *Massas e actividades dos moluscos*

Copo n.º	Data da colheita	Massa da concha (mg)	Actividade (c. p. m)			Actividade específica (c. p. m. / mg)		
			Concha	Partes moles	Detritos	Concha	Partes moles	Detritos
1	20 / III	94,4	188 572	191 459	33 346	1 998	2 028	353
2	20 / III	100,1	—	202 272	—	—	2 021	—
3	20 / III	—	—	—	—	—	—	—
4	20 / III	113,2	—	—	—	—	—	—
5	20 / III	92,8	181 089	131 679	47 400	1 951	1 419	511
6	24 / III	91,0	505 357	—	13 203	5 553	—	145
7	24 / III	112,6	126 988	134 625	32 672	1 128	1 284	291
8	24 / III	107,7	379 699	120 315	16 999	3 526	1 117	158
9	24 / III	94,5	372 983	—	19 176	3 947	—	203
10	24 / III	112,3	465 683	72 293	3 604	4 147	644	32
11	26 / III	101,8	426 702	103 232	2 976	4 192	1 063	29
12	26 / III	102,9	351 940	51 109	2 425	3 420	497	24
13	26 / III	89,9	439 993	81 359	11 660	4 894	905	130
14	26 / III	129,5	385 000	78 072	1 442	2 973	603	11
15	26 / III	—	—	—	8 426	—	—	—
16	31 / III	—	—	—	16 681	—	—	—
17	31 / III	131,9	345 472	78 356	2 739	2 619	594	21
18	31 / III	188,3	445 189	50 403	891	2 364	268	5
19	31 / III	106,6	236 593	62 728	4 942	2 210	587	46
20	31 / III	123,9	—	36 537	1 965	—	295	16
21	8 / IV	—	—	—	440	—	—	—
22	8 / IV	123,4	301 719	82 488	610	2 445	668	5
23	8 / IV	129,8	322 053	65 108	377	2 933	502	3
24	8 / IV	89,3	395 853	47 948	589	4 433	537	7
25	8 / IV	—	—	—	—	—	—	—

Observações :

Início do ensaio : 17 / III.

Os valores omitidos correspondem a moluscos de que se fizeram autorradiografias ou cujas amostras se inutilizaram.

Todos os moluscos se encontravam vivos no momento da colheita, com excepção do n.º 25.

Para ter-se uma ideia da absorção do cálcio pelos *Australorbis* e sua distribuição entre a concha e a parte mole determinaram-se os

QUADRO 4 — Valores médios das actividades específicas (expressas em c. p. m. / mg)

Copo n.º	Data da colheita	Concha	Partes moles	Fezes	Partes moles mais fezes	Concha / partes moles
1 a 5	20 / III	1 975	1 823	432	2 156	1,18
6 a 10	24 / III	3 650	1 015	166	1 175	3,49
11 a 15	26 / III	3 870	767	49	816	5,29
16 a 20	31 / III	2 398	436	22	456	5,62
21 a 25	8 / IV	3 270	569	3	574	5,91

valores médios das actividades encontradas nestas, correspondentes aos diversos dias em que se fizeram colheitas de moluscos. Tais valo-

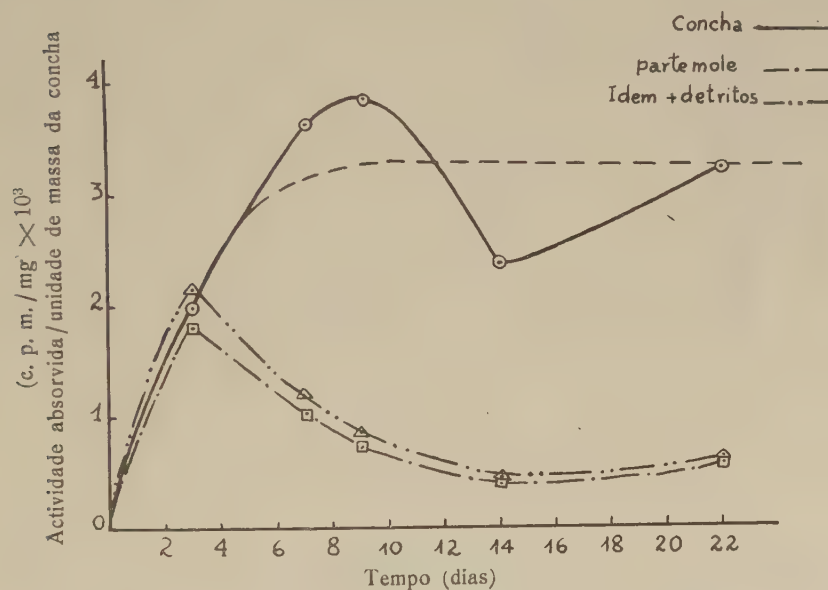


Gráfico 6 — Actividades das conchas e das partes moles

res encontram-se indicados no quadro 4, e com eles se construiu o gráfico 6.

Por este gráfico podemos concluir que, de início, a absorção de cálcio é rapidamente crescente quer na concha quer na parte mole, parecendo ser aproximadamente linear.

Cerca do quarto dia após o início do ensaio verificou-se que a actividade das partes moles decresce, primeiro rapidamente, depois

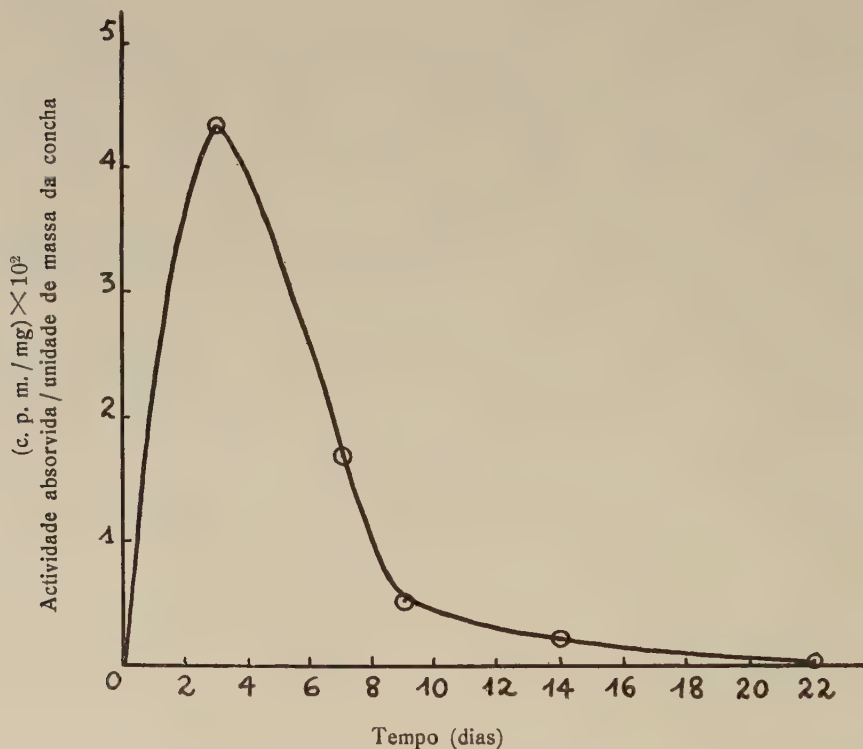


Gráfico 7 — Actividades das fezes

parecendo tender para um valor estacionário. Pelo contrário, as actividades medidas nas conchas continuaram a aumentar para além do quarto dia, parecendo também tender para um valor estacionário, indicado no gráfico 6 pela curva a tracejado. Julgamos poder atribuir a erros experimentais as anomalias verificadas na curva a traço contínuo. Deste modo, em face dos resultados obtidos, podemos admi-

tir que o *A. glabratus* absorveu cálcio do meio em que vivia e o distribuiu pela concha e parte mole.

Quando o teor em cálcio da solução passou a ser muito inferior ao inicial (gráficos 1 a 5), em virtude da absorção, o molusco deixou de poder manter o seu nível de cálcio à custa do meio exterior.

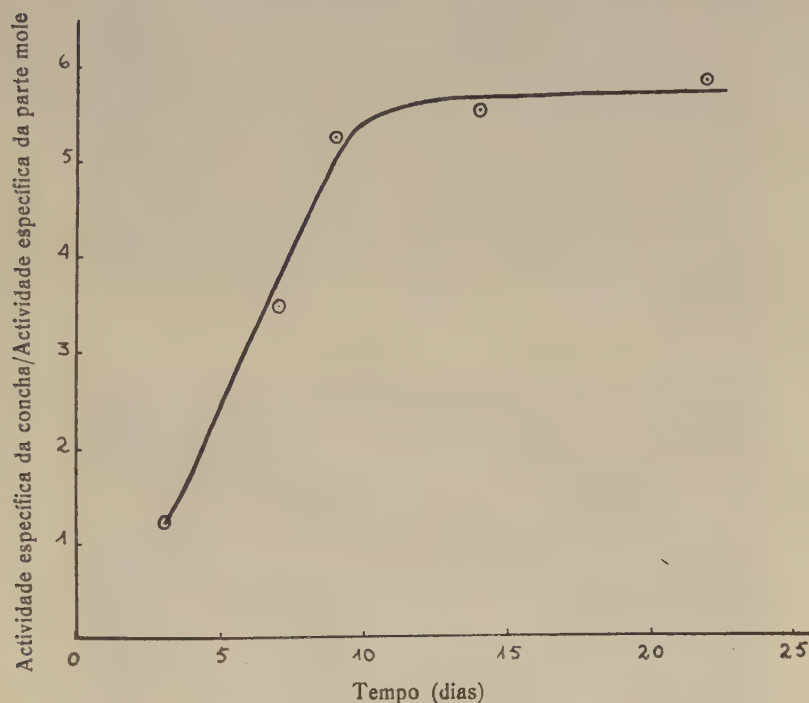


Gráfico 8 — Razão das atividades das conchas e das partes moles

Por conseguinte, a concha obteve o cálcio de que necessitou utilizando o da parte mole.

Estas conclusões são confirmadas pelos valores das atividades encontradas nas fezes, com que foi construído o gráfico 7.

O valor máximo desta actividade atingiu-se na mesma data em que as partes moles apresentaram os valores mais elevados. Note-se que o cálcio retido nas fezes se encontra numa forma química não solúvel na água, razão por que não é de novo absorvido.

Este conjunto de resultados permite inferir que o cálcio contido na concha deste tipo de moluscos é, pelo menos em grande parte, absorvido do meio líquido em que vivem, através das partes moles, que o transferem, por uma acção metabólica, para a concha. Atinge-se um valor estacionário na repartição do cálcio entre a concha e a parte mole; é o que se conclui das curvas de actividade existentes na concha e nas partes moles (gráfico 6) e o que é perfeitamente confirmado pelo gráfico 8, que nos dá a razão das actividades específicas existentes na concha e partes moles em função do tempo.

ESTUDO AUTORRADIOGRAFICO

De cada conjunto de cinco moluscos foi reservado um para estudo autorradiográfico.

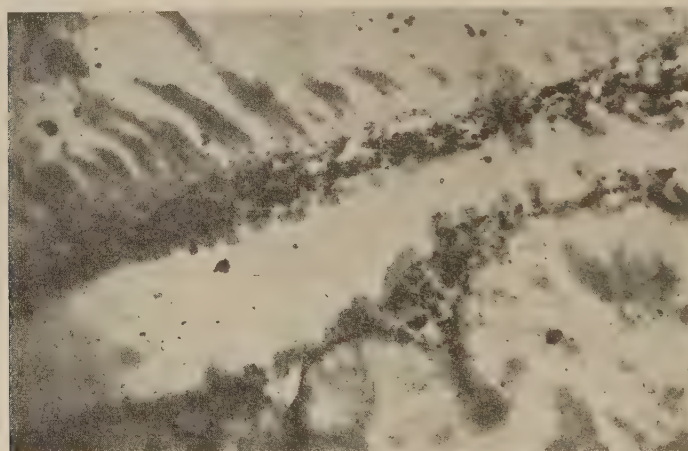


Fig. 1 — Fragmento do intestino, mostrando a distribuição do ^{45}Ca , particularmente nas vilosidades, por meio de película autorradiográfica

Para isso fizeram-se cortes histológicos das partes moles e a partir deles obtiveram-se, pela técnica de Pelc (11), microautorradiografias, nas quais se pode ver o ponteadado negro, correspondente à radiação beta do cálcio-45, localizado nalguns órgãos (figs. 1 a 5).

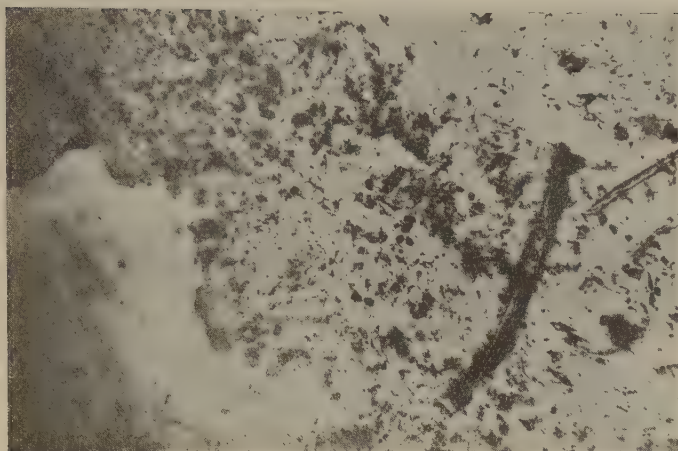


Fig. 2 — Corte de hepatopâncreas (com película autorradiográfica)

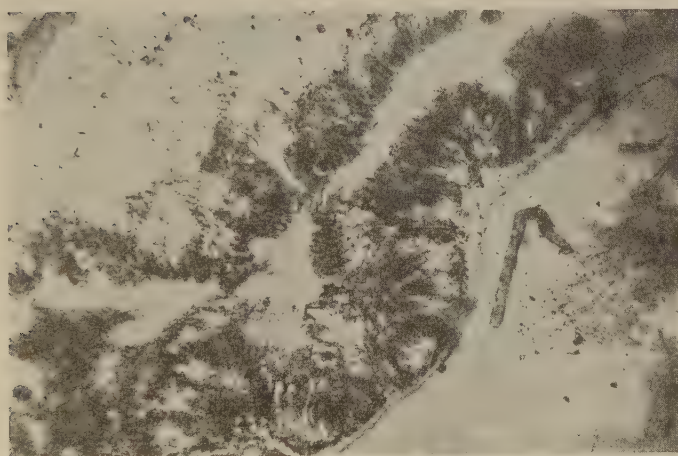


Fig. 3 — Glândula nidimental (com película autorradiográfica)

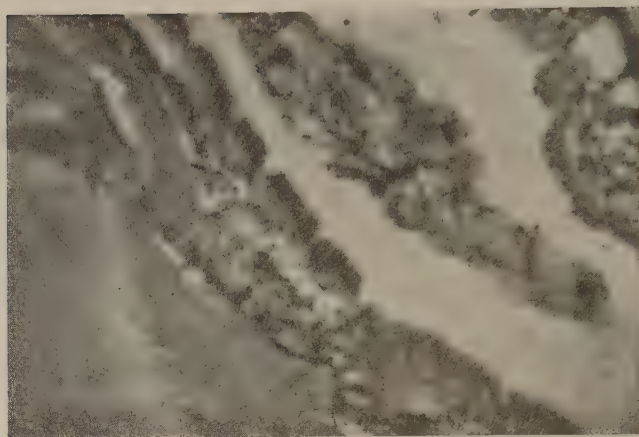


Fig. 4 — Fragmento do intestino e detrito alimentar, ambos com ^{45}Ca , revelado pela película autorradiográfica

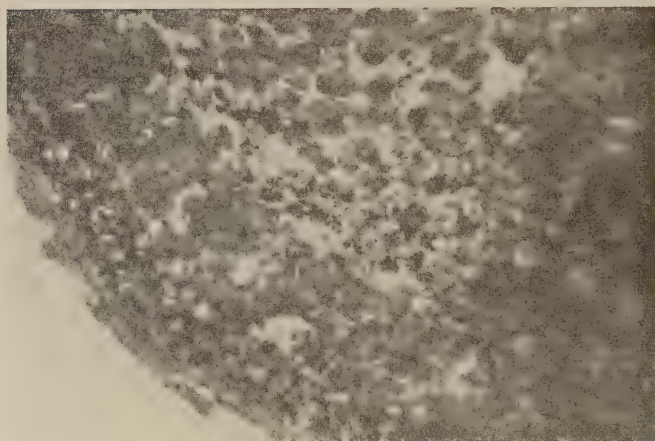


Fig. 5 — Fragmento de hepatopâncreas com abundante depósito de ^{45}Ca (com película autorradiográfica)

Em algumas lâminas não se colocou chapa autorradiográfica com o fim de servirem como testemunhas (figs. 6 e 7).

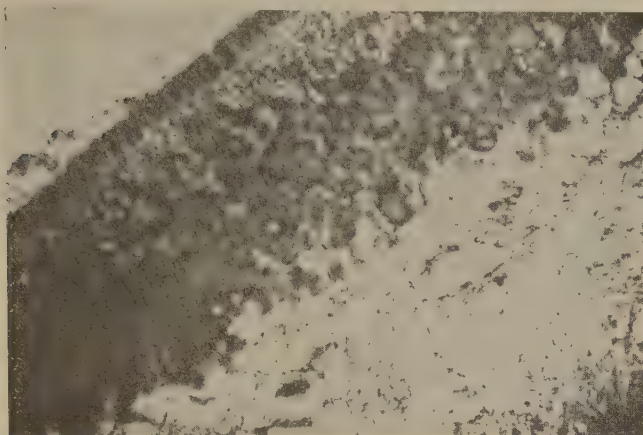


Fig. 6 — Fragmento de hepatopâncreas, em que se não vê o ponteadado negro correspondente à radiação emitida pelo ^{45}Ca , devido a ausência da película autorradiográfica (testemunha).

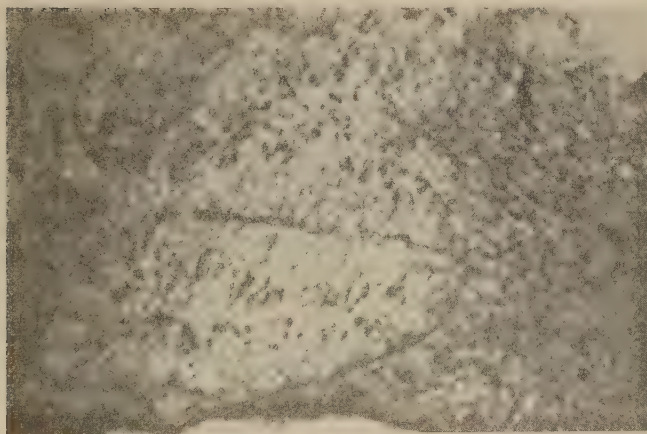


Fig. 7 — Glândula nidimental (testemunha)

Realizaram-se também macroautorradiografias, colocando o bloco de inclusão de parafina do respectivo molusco, depois de cortado, sobre película para radiografias dentais (figs. 8 a 12).



Fig. 8 — Macroautorradiografia das partes moles do molusco do copo n.º 3 (1.º lote); fraca absorção de ^{45}Ca

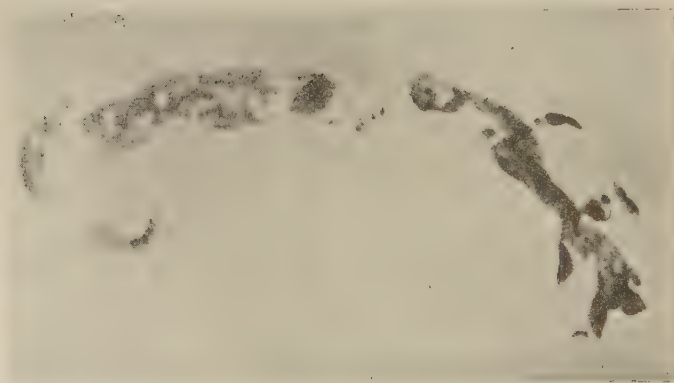


Fig. 9 — Macroautorradiografia das partes moles do molusco do copo n.º 6 (2.º lote); revela uma absorção de ^{45}Ca superior à anterior

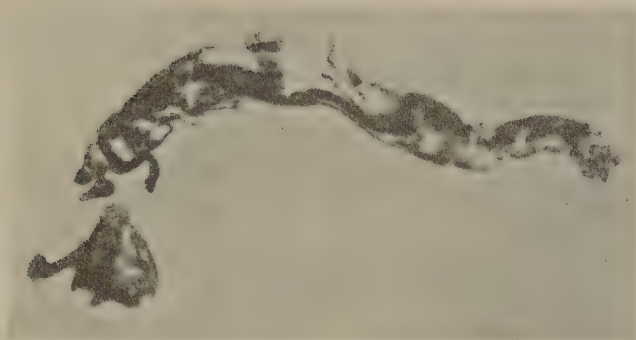


Fig. 10 — Macroautorradiografia das partes moles do molusco do copo n.º 15 (3.º lote); grande absorção de ^{45}Ca

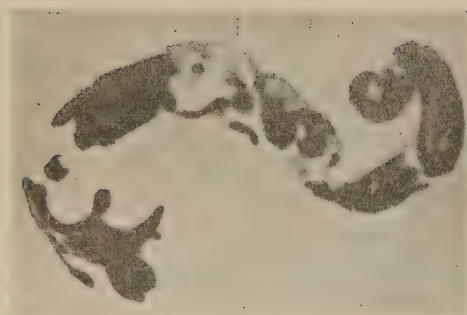


Fig. 11 — Macroautorradiografia das partes moles do molusco do copo n.º 16 (4.º lote); grande absorção de ^{45}Ca

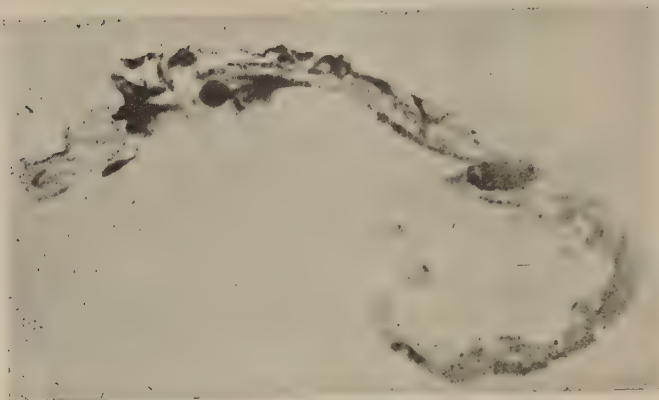


Fig. 12 — Macroautorradiografia das partes moles do molusco do copo n.º 21 (5.º lote); revela diminuição do teor de ^{45}Ca nas partes moles do molusco, o que está de acordo com as conclusões tiradas sobre a repartição do cálcio entre aquelas e a concha

Nestas últimas autorradiografias todo o corpo do molusco é visto em corte, em cada chapa, ficando impressionados nela os órgãos em que se encontrava localizado o isótopo utilizado neste estudo.

RESUMO

Os autores, prossequindo nos seus ensaios sobre o metabolismo de moluscos de água doce, estudaram a absorção do cálcio com o uso do isótopo ^{45}Ca daquele elemento.

Prepararam diferentes soluções radioactivas de ^{45}Ca e nelas colocaram moluscos adultos, da mesma geração, da espécie *Australorbis glabratus* (Gastropoda, Pulmonata); executaram medidas de actividade radiante das partes moles e das conchas respectivas, com vista ao estudo da distribuição daquele radioelemento pelas duas partes consideradas.

De alguns dos moluscos ensaiados fizeram preparações histológicas e subseqüente estudo autorradiográfico, que mostrou a localização especial do cálcio nos seus diferentes órgãos.

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A REMOÇÃO DE DEJECTOS E O ABASTECIMENTO DE ÁGUA NO CONTRÔLE DA ESQUISTOSSOMOSE

WALTER R. R. SANCHES ¹

O ciclo epidemiológico da esquistossomose permite que melhoramentos introduzidos no meio ambiente venham actuar como medidas eficazes de *contrôle* dessa enfermidade. Com efeito, a execução desses trabalhos — visando quebrar a cadeia ovo-miracidium-planorbídeo, ou objectivando evitar o contacto do homem com a água infestada pelas cercárias — é, sem dúvida, a medida profiláctica de maior alcance.

Vai daí a aceitação do conceito de que a disposição adequada dos dejectos humanos representa a primeira linha de defesa na profilaxia da esquistossomose. Em segundo plano, não menos importante, aparece a necessidade de se proteger o homem em relação à água, onde ele labuta ou dela faz uso, seja para a sua higiene pessoal ou recreação.

O estabelecimento do *contrôle* da esquistossomose nas bases das duas linhas citadas constitui, certamente, um pesado ónus para as administrações governamentais, principalmente tendo em vista que essa endemia grassa nas regiões de baixo padrão económico. Entretanto, não se pode pensar em debitar sòmente à campanha contra a esquistossomose todo o custo dos trabalhos; o alcance das medidas preconizadas extravasa os limites dessas campanhas e vai influenciar na redução da morbimortalidade por doenças entéricas, no desenvolvimento das comunidades e, consequentemente, na elevação do padrão de vida das regiões afectadas. Os benefícios oriundos desses traba-

¹ The author did not attend the meeting.

lhos devem ser medidos em termos económicos, e só neles se encontra a verdadeira avaliação.

REMOÇÃO DE DEJECTOS

Quase sempre a presença da esquistossomose se revela em áreas onde a instalação de sistemas públicos de esgoto sanitário não é justificada. Porém, mesmo nas comunidades desenvolvidas, assume importância o destino dado aos efluentes desses sistemas, especialmente se for verificada a presença de planorbídeo vector nas áreas por eles atingidas.

Quando é utilizado o processo de diluição para destino final do efluente, o problema perde significação. Entretanto, quando as águas receptoras são de volume reduzido, indispensável se torna um tratamento adequado.

Conhece-se hoje a sobrevivência dos ovos de *schistosoma* e dos *miracidia*¹ em relação aos processos usuais de tratamento dos esgotos sanitários. Sabe-se que os processos biológicos (lodo activado e biofiltração) permitem o desenvolvimento do ovo em *miracidium*; que o ovo é facilmente retido na filtração intermitente, enquanto esse processo permite a passagem dos *miracidia*; que a sobrevivência do ovo nos tanques sépticos, digestores e leitos de secagem do lodo é função do tempo e da temperatura. De posse desse conhecimento, pode o engenheiro projectar instalações adequadas para o tratamento.

Nas comunidades não servidas por sistemas de esgoto sanitário, ou mesmo na periferia das cidades (onde não se justifica a instalação da rede colectora), lança-se mão de instalações particulares. Dentre essas as mais frequentes são o tanque séptico — para habitações de padrão mais elevado — e a «privada higiênica» ou «fossa seca». Reconhece-se que em ambos os casos a solução é temporária; porém, objectiva-se, assim, enquadrá-la nas condições socio-económicas locais.

¹ «The Effector Sewage Treatment Processes upon Ova and Miracidia of *Schistosoma japonicum*», by W. L. Newton, W. B. Foggat and S. B. Weibel, *Sewage Works Journal*, Vol. XX (4), 1948, p. 657.

O tanque séptico, sendo quase que de tipo padronizado, não merece referência especial. A sua aplicação se limita às casas que dispõem de instalação hidráulica domiciliária e têm aceitação generalizada.

Base de madeira em bruto com piso de varas e barro



- 1 — As varas devem ser rectas e resistentes
- 2 — As varas devem ser colocadas com cuidado para deixar o mínimo espaço entre elas
- 3 — Depois de colocar as varas como mostra a figura A, põe-se em cima uma camada de barro de 5 cm, dispondo-se a seguir outras varas atravessadas, como mostra a figura B
- 4 — A abertura deixada no centro do piso terá 20×40 cm

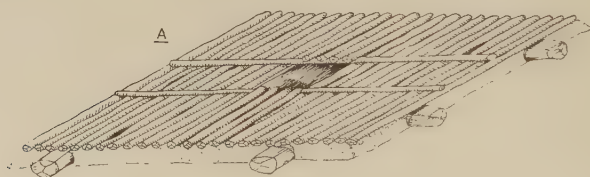
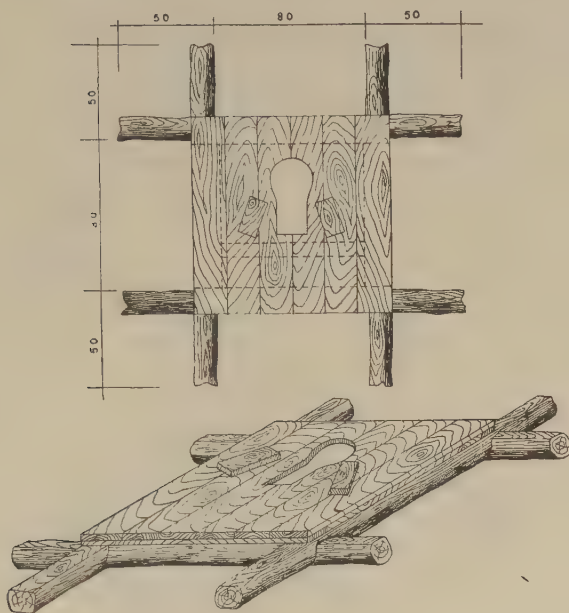


Fig. 1

Já a «privada higiênica» precisa atender às peculiaridades de cada área, como sejam a disponibilidade de materiais locais, o poder aquisitivo da população e os hábitos e costumes regionais.

Essas diferenças se manifestam, principalmente, no tipo da laje usada na cobertura do buraco.

Base de madeira em bruto e piso de tábuas



- 1 — As tábuas devem ser de madeira resistente
- 2 — O piso não deve ter fendas
- 3 — É aconselhável pregar uma tábua em cima de cada pau da base, procurando nivelá-los, facilitando, desse modo, o assentamento das tábuas do piso
- 4 — Depois de as tábuas estarem pregadas, um montículo deve ser construído para proteger o buraco contra as águas de enxurrada

Fig. 2

Algumas têm o assento elevado, com ou sem tampa, e de forma variada; outras têm apenas uma abertura e dois pequenos ressaltos para apoio dos pés; algumas têm sifão e são descarregadas com auxí-

lio de água; podem ser elas de madeira, de taipa ou de concreto. É essencial que o tipo escolhido tenha aceitação por parte do povo e que seu custo seja compatível com as condições locais.

Outros factores podem ainda intervir na selecção do tipo de laje. É o caso, por exemplo, da laje de concreto armado quadripartida,

Laje ideal para piso de privada higiénica

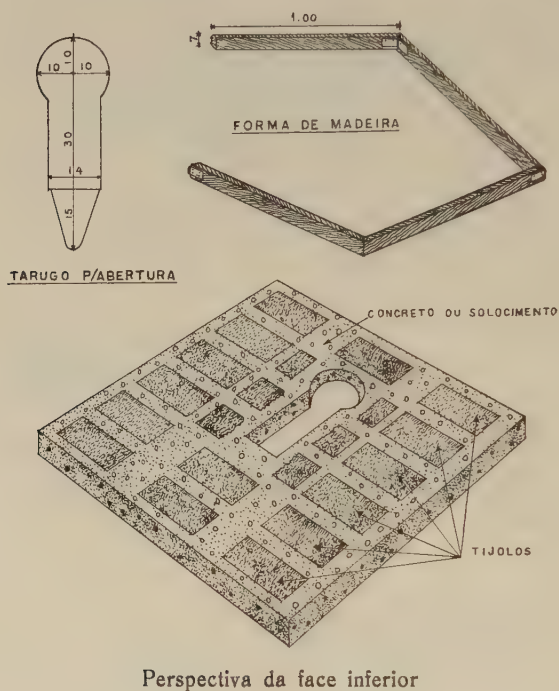


Fig. 3

utilizada quando se recomenda a sua produção centralizada e os pontos de aplicação são de difícil acesso. Nesse caso o próprio interessado pode transportá-la pessoalmente, pois cada uma de suas partes pesa apenas 18 kg.

No que diz respeito ao financiamento de tais instalações, é de toda a conveniência a participação directa do beneficiado, seja atra-

vés do fornecimento de materiais, seja pela aplicação do seu esforço pessoal na construção. É essa uma fórmula que actua no sentido de

**PISO PARA PRIVADA HIGIÉNICA
CONSTRUIDO EM 4 PARTES**

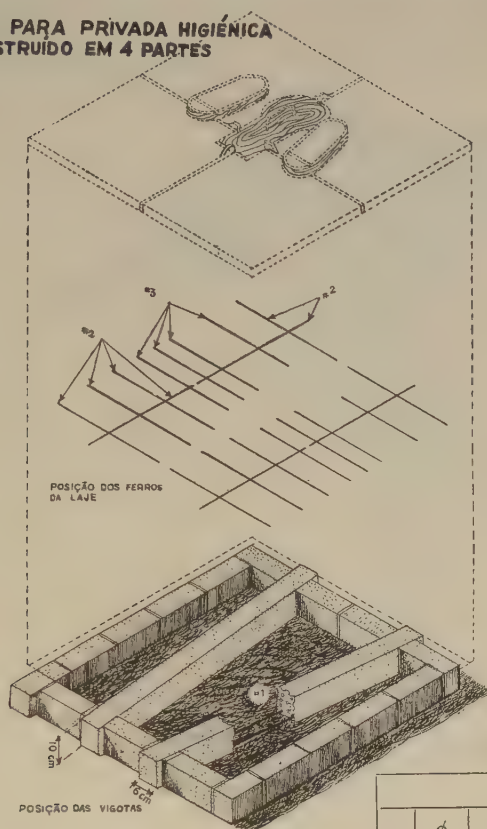


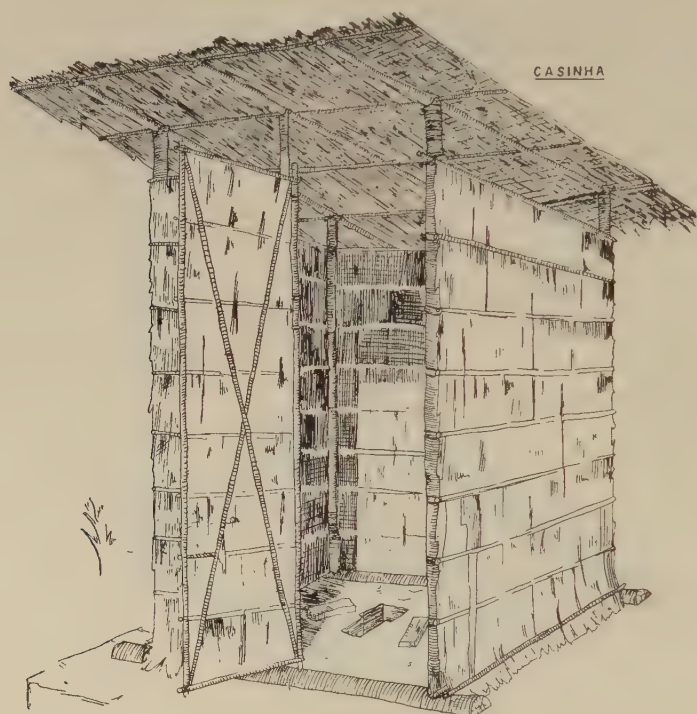
Tabela de ferros							
#	Ø //	Quant.	Comprim. (m)		Peso (kg)		Uso
			Unit.	Total	Unit.	Total	
1	3/16	4	0,96	3,84	0,13	0,54	Vigotas
2	3/16	12	0,47	5,64	0,07	0,29	Laje
3	3/16	8	0,37	2,96	0,05	0,42	
Totais . .		24	—	12,44	—	1,25	—

Fig. 4

educar o povo para a manutenção da instalação, através do reconhecimento dos valores nela investidos.

ABASTECIMENTO DE ÁGUA

Existe hoje, de maneira quase generalizada, uma forte demanda popular pela instalação de sistemas públicos de abastecimento de



- 1 — A casinha será coberta com palha
- 2 — Cada camada consistirá de 4 palhas, colocadas 2 por dentro e 2 por fora, amarradas fortemente com cipó
- 3 — Na cobertura haverá um beiral de 30 cm em todos os lados
- 4 — Deverá existir uma abertura de ventilação de 10 cm entre as paredes e a cobertura

Fig. 5

água. Essa demanda, decorrente do conforto que esses sistemas propiciam, pressiona os órgãos governamentais, que, de imediato, se vêm à frente do problema do financiamento das obras.

As soluções clássicas para financiamento desses sistemas não podem encontrar guarida onde o subdesenvolvimento predomina. Se nos países de economia estabilizada e de elevada renda individual o financiamento é feito com a participação ponderável e directa dos individuos — através de títulos de dívida pública, apólices de orga-

Privada para lugar alagado

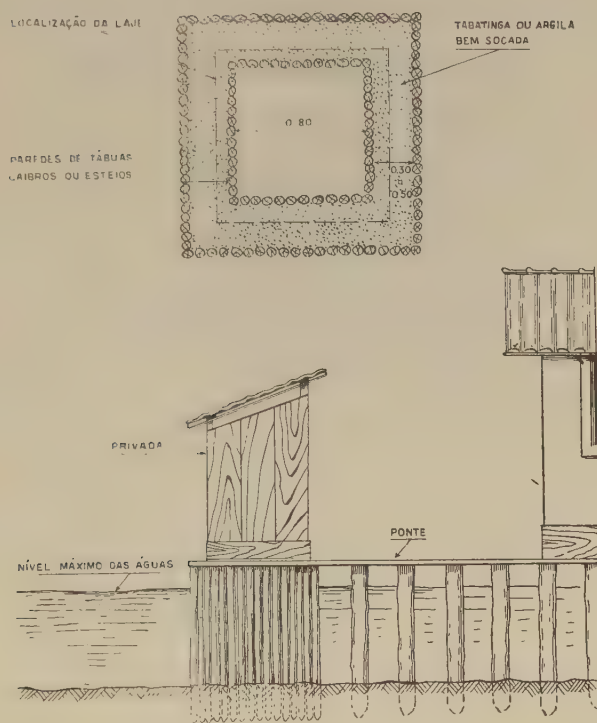


Fig. 6

nizações de economia mista, etc. —, já naqueles econòmicamente imaturos é praxe a doação dos serviços às municipalidades por parte dos governos centrais. Considerando, entretanto, a simultaneidade e o número elevado de solicitações feitas para a instalação desses sistemas, é óbvio que as mesmas não podem atendê-las em prazo razoável.

É, geralmente, nesse segundo caso que recaem as áreas flageladas pela esquistossomose e, assim sendo, é preciso buscar nova fórmula de financiamento. Essa fórmula deverá repartir, equânimemente, os encargos financeiros em função dos benefícios auferidos e da capacidade financeira de cada um. Além disso, cumpre observar as responsabilidades delegadas, pela constituição de cada país, aos níveis

Privada com sifão

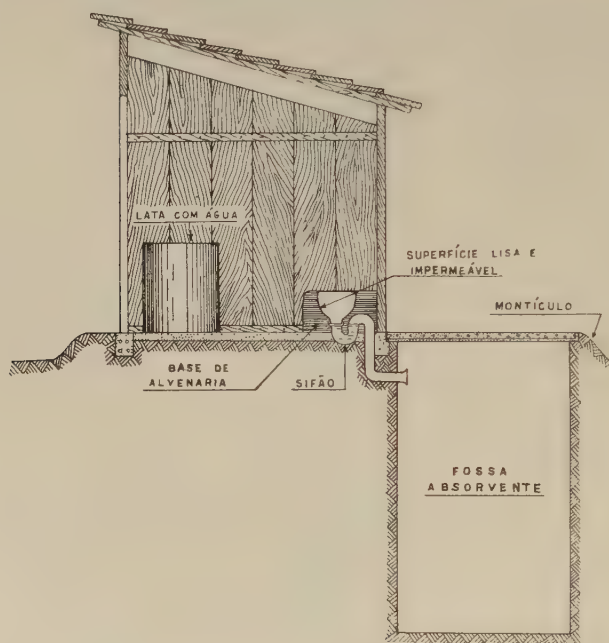


Fig. 7

administrativos local, regional e central, no que tange ao abastecimento de água das comunidades.

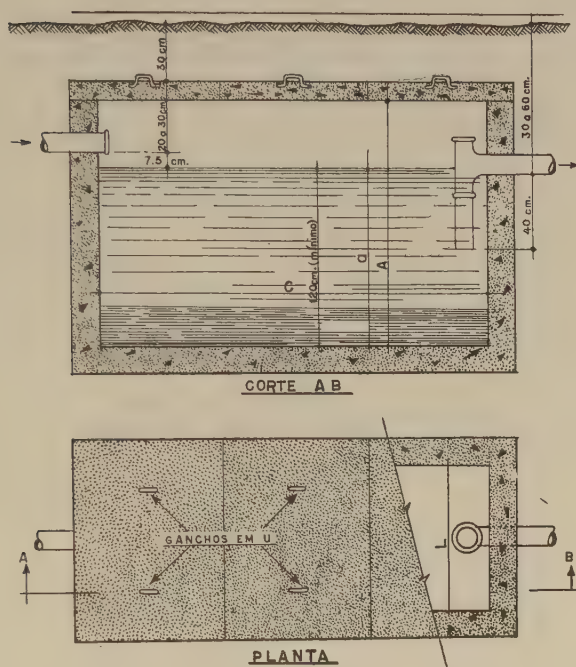
Em seguida à questão do financiamento surge o problema da operação e administração dos serviços instalados.

É esse um ponto sobre o qual o engenheiro projectista deve dedicar especial atenção. Sem sacrificar a técnica, precisa ele de simpli-

ficar as instalações de molde a permitir que as exigências mínimas de operação e manutenção sejam compatíveis com a capacidade técnica e econômica do órgão dela encarregado.

Tanque séptico

Padrão para pequenos tanques (até 10 pessoas)



Os números da figura são constantes para qualquer tamanho de tanque

Fig. 8

No que diz respeito à administração dos serviços instalados, é de gravidade o problema nos países de economia debilitada. A carência de pessoal especializado e de nível de instrução adequado para o trabalho tem submetido o problema a inúmeras tentativas de solução mais ou menos felizes.

A título de exemplificação, veja-se o que se passa no Brasil: Pela Constituição Federal, é da responsabilidade da municipalidade o problema do abastecimento de água. Acontece, porém, que a arrecadação municipal é irrisória em face às rendas estaduais e federais, do que resulta o sistema de doações desses últimos níveis admi-

Pormenores da lavadaria

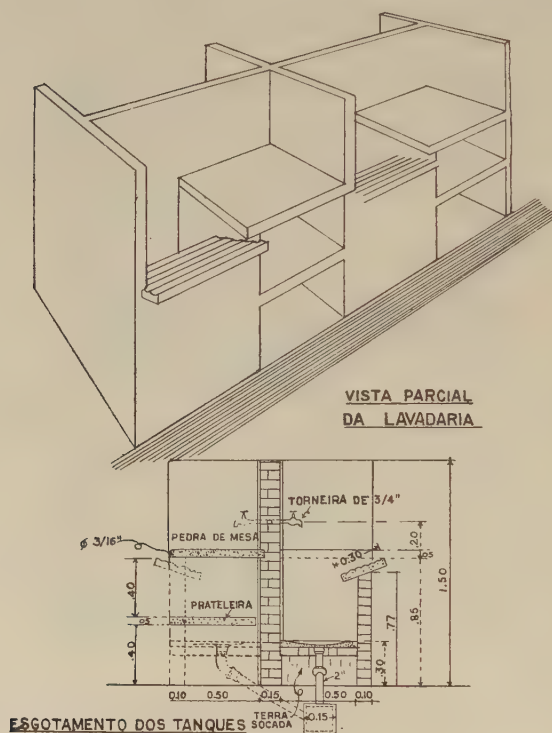


Fig. 9

nistrativos para o primeiro. Além disso, a concentração dos elementos técnicos nas grandes cidades, pela falta de atractivos oferecidos pelas pequenas comunidades do interior, concorre, praticamente, para a transferência das responsabilidades estabelecidas na Constituição. Surge, então, o expediente dos «convênios», mediante os quais as

obrigações municipais são transferidas, por determinado periodo de tempo, para os níveis estadual e federal.

No estado do Rio Grande do Sul o governo estadual financia, mediante a assinatura de convênios, a construção dos sistemas e, como compensação, se encarrega da administração dos serviços, inclusive

Pormenores da lavanderia

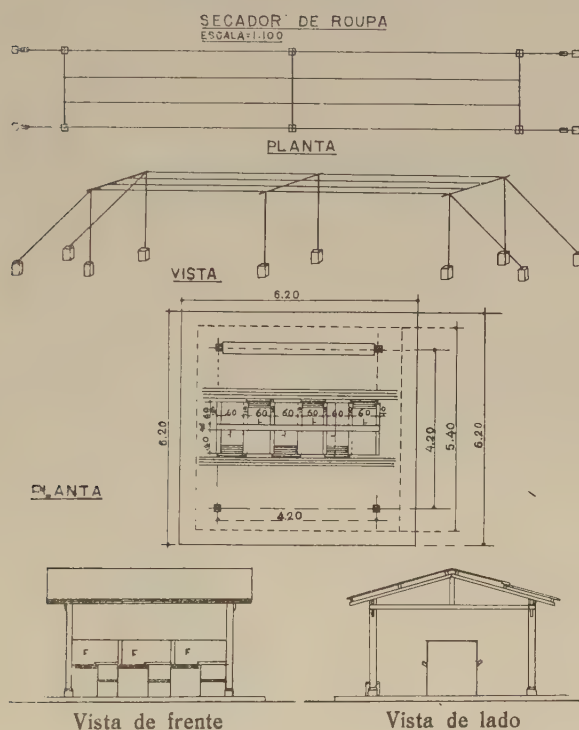


Fig. 10

para arrecadar as taxas de água, a título de pagamento dos empréstimos feitos e de cobertura das despesas de operação.

Esse sistema apresenta como vantagem uma administração de bom padrão. Por outro lado, o município nunca se liberta dos pagamentos dos empréstimos feitos (as taxas de água são baixas e uni-

formas, por exigências burocráticas) e, assim, jamais se preparará para assumir os encargos que lhe cabem. Acrescentem-se ainda os

Fases da construção

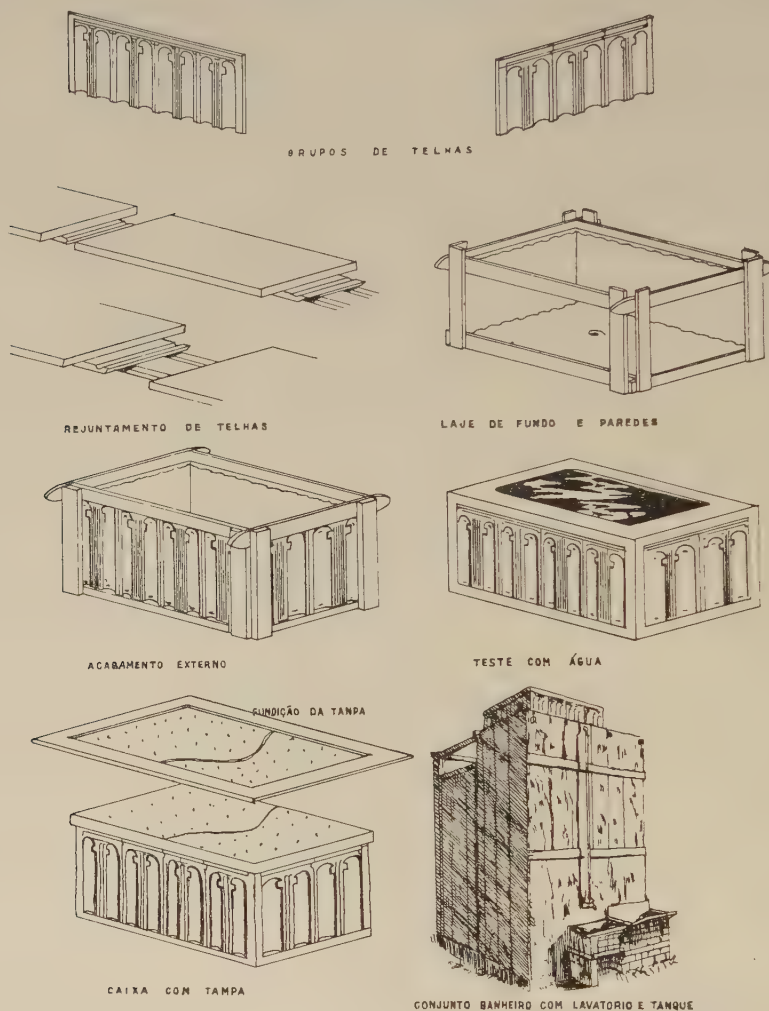
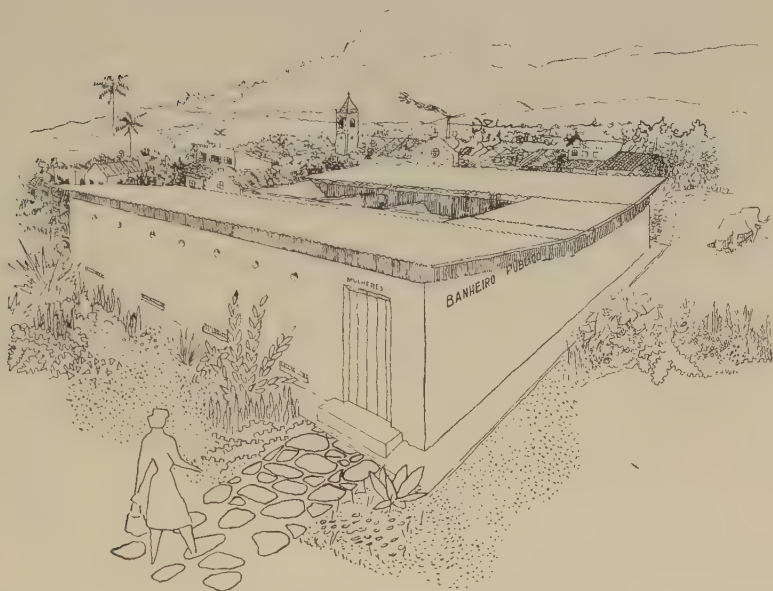


Fig. 11

ônus a que se obriga o estado para manter um órgão em proporções necessárias à cobertura de todas as suas cidades.

Procurando contornar essas dificuldades, está em aplicação, pelo Serviço Especial de Saúde Pública (SESP), órgão federal do Ministério da Saúde, uma outra fórmula, a qual consiste em fazer criar, pela municipalidade, um órgão autárquico para a administração e operação do serviço de abastecimento de água. A administração desse órgão é, então, contratada com o SESP. Assim, com apenas um

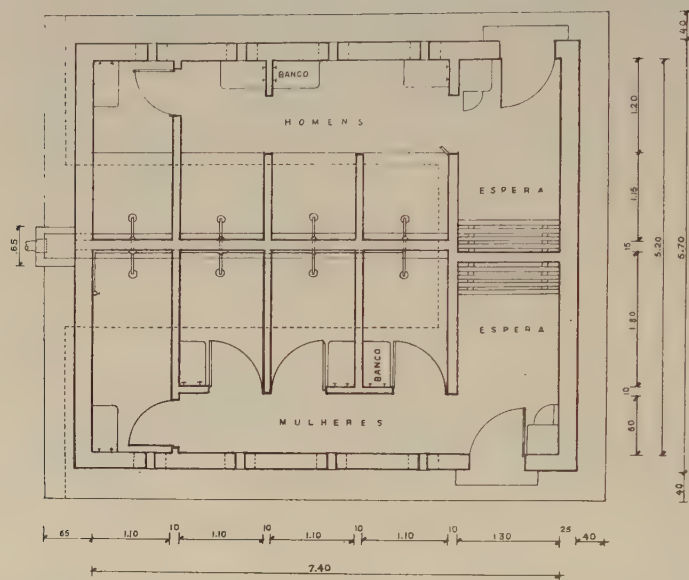
Banheiro público



Perspectiva

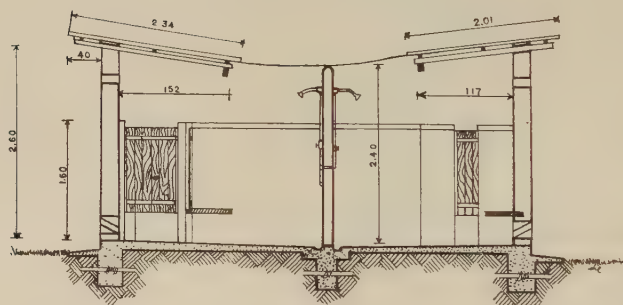
Fig. 12

engenheiro, um mecânico e um auxiliar de administração, pode o SESP dar cobertura a uma área com várias cidades, pois que os demais elementos são locais e pertencem aos serviços onde trabalham. Outra vantagem oferecida por essa fórmula é que com isso cria-se, para o município, um órgão próprio constituído por elementos locais, de padrão razoável pelo treinamento que recebem do SESP.

Banheiro público

Planta

Fig. 13

Banheiro público

Corte

Fig. 14

Já no estado de São Paulo, de padrão económico relativamente elevado, as municipalidades constroem seus próprios sistemas com empréstimos que levantam nas organizações bancárias e se responsabilizam, directamente, pela administração e operação dos serviços instalados. Ao estado cabe apenas aprovar os projectos das obras, quanto aos seus aspectos técnicos, caso o financiamento seja concedido por órgão oficial ou officioso.

No que diz respeito especificamente ao *contrôle* da esquistossomose, procura-se nos sistemas instalados trazer a água para a proximidade do indivíduo e, assim, evitar que ele vá ter às águas contaminadas.

Dentro desse objectivo são construídas bicas ou torneiras públicas, localizadas em pontos estratégicos, nas quais o indivíduo poderá buscar a água de que necessita para o seu uso. Também as lavadeiras e banheiros públicos permitem ao povo exercer um mister e satisfazer a sua higiene pessoal.

Procurando facilitar ainda mais ao indivíduo a utilização da água não contaminada, devem ser desenvolvidos reservatórios, banheiros e tanques domiciliários rústicos, feitos com material de fácil obtenção e compatível com as suas posses.

A título de ilustração, os desenhos anexos apresentam alguns tipos dessas instalações em uso no Brasil.

ON THE DISTRIBUTION AND BIOLOGY OF DERMATITIS-PRODUCING SCHISTOSOMES IN WESTERN NORTH AMERICA¹

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Miller (1925 and 1927) found *Cercaria elvae* in *Lymnaea stagnalis* at Trout Lake, San Juan Island, Washington, in the northwest corner of the United States of America, before it had been implicated with the production of dermatitis. Macfarlane and the writer (1946) first experimentally produced schistosome dermatitis in western North America, implicating *Cercaria oregonensis*, a new species, as the etiological agent. The intermediate host was demonstrated to be *Physa ampullaria*, collected in ponds in northwestern Oregon. One case of schistosome dermatitis has been reported as originating at Blue Lake, near the Columbia River, on the northeast boundary of Portland. Infected *Physa* snails also were collected in an arm of Lake Oswego, several miles southwest of Portland. A temporary pond at Milwaukie, several miles south of Portland, furnished many infected snails and produced a number of experimental cases of dermatitis. An alkaline pond near Tygh Valley, in the arid sage-brush country, one hundred miles east of Portland, yielded *Physa* snails infected with schistosomes. Cases of dermatitis associated with swimming in a lake at Albany, 75 miles south of Portland, have been reported to the author.

¹ This work was supported by Contract Nonr 1805(00), NR 130-342, between the Office of Naval Research, Department of the Navy, and Portland State College.

A case of dermatitis in a logger so severe that it resulted in hospitalization was acquired in 1956 from a log pond at a lumber mill at Carlton, about 35 miles southwest of Portland. A survey made in December of *Physa* snails in this large pond revealed that approximately 20 percent were infected with the cercaria of *Trichobilharzia oregonensis*. This is a first report of what may be termed «logger's itch».

Hunter *et al.* (1949) reported *Cercaria elvae* as the cause of the severe dermatitis which resulted in the closing of Green Lake, Seattle, Washington, to swimming. The writer (1952 and 1953) proved that the etiological agent of schistosome dermatitis at Cascade Lake, on Orcas Island, Washington, also was *Cercaria elvae*. Schistosome dermatitis is very common both in western Washington and also across the Cascade Mountains, in the more arid part of the state; it is also a problem at the popular resort area of Wauna Lake, in southern Washington. Dr. Ethel Reuter, public health officer, informed me that her son was scarcely able to swim in the lake because of the resultant dermatitis.

Recently, Farr and Blankemeyer (1956) have discovered a new species of *Trichobilharzia*, which they named *T. brantae*, in the Canada Goose. The species most nearly resembled *T. physellae*, but since the larval stages of *T. brantae* were not described, the interesting question arises as to whether reports of *T. physella* cercariae from Oregon and Washington might be referred to *T. brantae*. Some of the specimens of *T. brantae* came from a goose collected on the Turnbull National Wildlife Refuge, in the state of Washington, whereas most of the records are from geese taken in states on the East Coast.

Various recent publications from California indicate that schistosome dermatitis occurs in various widely separated areas of that state. Mortenson and Mulhern (1951) discovered the malady in a Sierra Nevada lake, and Walker (1954) discovered cases at Twain Harte Lake, Tuolumne County. Simmonds, Martin, and Wagner (1951) proved that cases of dermatitis which followed exposure to the water of an artificial lake in Alondra Park, in Los Angeles, were due to schistosome cercariae released from *Physa osculans*.

Penner (1950) has described a cercaria from the common intertidal snail *Littorina planaxis* from Bird Rock, near La Jolla, California,

and from the Coronado Islands of Mexico, which is capable of producing dermatitis. Reports from other localities are being investigated.

Jellison *et al.* (1952a) refer to three localities in Montana where severe dermatitis has occurred and they implicated *Lymnaea palustris* and *L. stagnalis* as intermediate hosts.

Edward and Jansch (1955) studied an outbreak of schistosome dermatitis at Cultus Lake, British Columbia, and as a result they discovered two new species of schistosome cercariae emerging from *Physa* snails. One species was reared to maturity in ducks and was identified as *Trichobilharzia adamsi*. Localities of schistosome dermatitis in Saskatchewan and Alberta have been reported to the writer by public health officials of those provinces.

Most interesting is the discovery by Jellison *et al.* (1952b) of an outbreak of schistosome dermatitis at Beaver, Alaska, which is only six miles south of the Arctic Circle. A group of people who went swimming in a pond known as «Itch Slough», a mile east of Beaver, were severely affected. Numbers of *Lymnaea palustris* from this water course shed large numbers of schistosome cercariae and the writer, after examining prepared slides of the material, identified them as members of the genus *Trichobilharzia*. Subsequent studies by Harkema (1953 and 1957) have demonstrated that schistosome cercariae are widely distributed in central Alaska. He reared material in ducklings and described the worms as a new species, *Trichobilharzia alaskensis*.

During a trip to Alaska in 1956, the writer learned that at certain times gravel-pit ponds near Fairbanks could not be used for swimming because of heavy infestation with schistosomes. An examination of certain snails in the vicinity of Pt. Barrow did not yield any trematode larvae.

It is now clear that schistosome dermatitis and a variety of causative agents, both fresh water and marine, are abundantly distributed in western North America from Mexico to central Alaska. Much, however, remains to be learned about details of distribution, taxonomic relations, life histories, and epidemiology of the schistosomes involved.

LIFE HISTORIES OF DERMATITIS-PRODUCING SCHISTOSOMES
IN NORTHWESTERN UNITED STATES AS ILLUSTRATED BY
MOTION PICTURES

A typical case of schistosome dermatitis acquired by a student who served for a time as my research assistant is shown first. Repeated exposures to the cercariae increased his sensitivity to the organisms until he no longer was able to work on the project. The next case is also of a boy who aided me in collecting snails.

Next are seen several views of living cercariae of *Trichobilharzia oregonensis*, which commonly occur in lakes, ponds, swamps, and slow-moving streams. Next is seen the large, widely distributed snail *Lymnaea stagnalis*, host of *T. elvae*, in the state of Washington, and other northern states and Canadian provinces.

Now some experimental methods of studying the biology of these schistosomes are introduced. Snails to be examined for infections are isolated in medium-sized shell vials arranged in small wooden trays which are easily held up to the light for inspection.

To learn the best method of infecting hatchery-reared ducklings, some were placed in a glass container in such a manner that only the feet could come in contact with schistosome cercariae. A rubber dam prevented the birds from drinking the water. The other method consisted of administering *per os* quantities of cercaria-infected water. Cercariae from several snails were mixed in order to insure the presence of both sexes. Results indicate that both methods of infection were very effective.

Between two and three weeks after exposure, the ducks were opened and intestine removed, as shown here. Pieces of intestine were slit open and pressed between two 2×3 inch glass slides, after which a search under the binocular dissecting microscope was carried out. This scene shows adults of *T. oregonensis* located inside the smaller veins, especially those on the serosa side of the intestinal wall. The lighting is insufficient because of the thickness of the intestinal wall; however, it is possible to locate the worms because of the dark, undulating intestine. It should be mentioned that the more delicate *T. elvae* adults tend to be found in small veins on the mucosa side of the intestine.

This is a view of a living male *T. oregonensis* obtained by injecting the hepatic portal vein with citrate solution and then withdrawing it. Total length of the adult male is generally between 5 mm and 6 mm whereas the female is only slightly smaller. The expanded central part of the gynecophoral canal is about four times as long as that of *T. elvae*. Movement of the acetabulum is demonstrated.

Eggs *in situ* in the intestine of the duck host are seen to have a nearly straight axis. They are not uniformly distributed but tend to be locally concentrated.

The next scene shows an egg with miracidium about ready to hatch. It has been dissected from the intestinal wall and moves actively after the egg is placed in water. A second miracidium is also shown escaping from an egg. It will be noted that emergence here is from the blunt end.

Another view shows the eggs of *Trichobilharzia elvae* found in northern Washington and also eastward across the continent. The eggs of this species are quite different and possess a strongly curved axis, similar to those of *T. ocellata* of Europe. The phase contrast system of the microscope increases the visibility of structures. Eggs that remain in the intestinal wall, even for a rather brief period, produce an inflammatory reaction.

In order to recover miracidia, between 5 g and 10 g of feces of infected ducks were placed in a McMullen-Beaver flask of 540 cm³ capacity. As can be seen, this flask is made opaque with black paint except for a small side arm toward which a light is directed. Since the miracidia are positively phototactic, they invariably swim into the side arm and are removed with a pipette.

We see a row of such flasks maintained at about 30°C, at which temperature hatching occurs in a minimum time of 20 minutes and a maximum time of 80 minutes.

The next scenes are of newly hatched miracidia, restrained by pressure of the cover glass. The phase-contrast system better shows such structures as cilia.

Now several miracidia are introduced into a vessel containing a very young *Physa*, in which the shell is still translucent. The miracidia are observed passing at high speed through the field and at first give little evidence of reacting to the snail's presence. Eventually, however, one or more of them contacts the snail and penetrates.

The branching sporocyst from the liver of a crushed snail shows the great amount of parasitic growth which may occur in this host. Cercariae are observed both inside and outside of the sporocyst. Large numbers of discharged cercariae are seen in the vicinity of another crushed snail. In laboratory trials large numbers of cercariae of *T. oregonensis* were found to issue from late afternoon to evening, but they also may emerge in the morning or at other times, depending upon variable factors.

The author's house-trailer field laboratory, next shown, has been of special value in allowing for efficient investigations at sites distant from headquarters at Portland State College.

Much investigation of *Trichobilharzia elvae* from *Lymnaea stagnalis* was carried out at Cascade Lake, Orcas Island, Washington. Swimmers at one of the two bathing beaches of this lake are shown about to plunge into the schistosome-infected waters.

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GENERAL DISCUSSION

DR. JOSEPH E. AZAR (Lebanon) — Perhaps the comment I like to make now seems to be more related to certain points that were raised in yesterday's discussion on the subject of diagnostic procedures in schistosomiasis. In view of the relative importance of the rectal biopsy technique in the diagnosis of a schistosomal infection, I feel that it is justifiable to state a few words on this procedure, more so that I could see no reference made to it in the papers on the subject under discussion.

What I shall be saying makes no news to you, for Ottolina first and others in the Americas and elsewhere have repeatedly reported on the significance of this procedure and its advantages over the orthodox methods of diagnosis, namely, urine examination in *S. haematobium* and feces examination in *S. mansoni* infections. After having some personal experience with the rectal biopsy technique and the excellent results it gives, both in the field and in the clinic, I feel convinced that more use of it should be carried out in routine practice.

With my friend Dr. R. J. Pitchford, who has a far wider experience with the procedure, we were able to diagnose all *S. mansoni* infections and practically all *S. haematobium* ones in some South African Bantu children in a rural area where infection with both species of the trematode (*S. mansoni* and *S. haematobium*) were more or less equally prevalent. The rectal biopsy was found to be positive for both *S. mansoni* and *S. haematobium* ova in the same individual harboring a mixed infection. Incidentally, only the *S. mansoni* ova were found to be viable, whereas the *S. haematobium* ones were black, non-viable, a peculiar condition to which we have no explanation.

Therefore, in the field, more so in the presence of mixed infections, the rectal biopsy technique offers a very useful tool in the diagnostic survey of the infection. Similarly, in the clinic this procedure ought to be the method of choice for the diagnosis of an

established *S. mansoni* infection, rather than the unreliable ordinary microscopic examination of a fecal specimen. It is an easy procedure to perform, quite a practical one, and yields very reliable results. Thus its more general use is to be highly recommended. I hope that, if time permits, others will as well comment on this diagnostic procedure in schistosomiasis.

DR. ALINA P. SZUMLEWICZ (Brazil) — The superiority of Molluscicide Bayer 73 was shown as referred to the concentration used and as far as fish killing is involved.

I would like to know whether any biological tests on the residual effect of this compound have been done.

DR. DURVAL DE LUCENA (Brasil) — A ideia do *contrôle* da esquistossomose pelos moluscocidas é impraticável no momento, dadas as enormes extensões territoriais onde vivem os caramujos a tratar. Daí, em minha opinião, deverem ser activadas as pesquisas no sentido de se obter um medicamento de utilização oral, com que, ao mesmo tempo, se possa curar o doente, destruindo o parasita e evitando a continuidade do ciclo biológico.

DR. ALICE BUTTNER (France) — À propos du produit Bayer 73 (molluscicide), quel est son comportement dans des eaux faiblement courantes? Les qualités du taux bas de solubilité, stabilité, non toxicité, vis à vis de la faune peuplant le biotope des mollusques sont-elles complétées par le poids de ce produit, lui permettant de persister plus longtemps dans les marigaux fréquentés par les populations de gastropodes, hôtes intermédiaires des bilharzies humaines.

DR. TITO DE MORAIS (Portugal) — O Instituto de Investigação Médica de Moçambique terá todo o interesse em realizar os ensaios de campo do Bayer 73, em colaboração com o Dr. Gönert, na área de irrigação do vale do Limpopo.

DR. JEAN SCHNEIDER (France) — La communication du Dr. Friedheim m'incite à préciser deux points:

1. *Tolérance*. — J'ai eu le privilège, il y a plus de 3 ans, d'être un des premiers utilisateurs du TWSb; j'ai constaté une excellente

tolérance générale; comme tout médicament antimonie, le TWSb, à partir d'une certaine dose, provoque des modifications de l'électrocardiogramme (aplatissement de T — allongement de ST); ces changements sont plus des témoins de la présence et de l'accumulation de l'antimonie que des signes d'atteinte du myocarde; le retour à la normale de l'électrocardiogramme s'observe 1 mois à 6 semaines après la dernière injection de TWSb. J'estime que ce retour à la normale est la plus sûre indication autorisant la reprise éventuelle d'un traitement antimonie si l'état du malade justifie une normale cure.

2. *Activité.* — Je considère le TWSb comme le meilleur médicament actuel de la bilharziose; il n'empêche que j'ai observé un échec après un traitement administré aux doses indiquées par Friedheim — cet échec est survenu chez un malade traité pour bilharziose à *S. haematobium* à Paris, non soumis à des reinfestations 12 mois après la fin du traitement. Les examens répétés des urines étaient restés négatifs pendant 11 mois consécutifs lorsqu'au 12ème mois j'ai retrouvé à plusieurs examens des œufs viables de *S. haematobium*; cette «rechute» tardive est, à mon avis, un argument de plus en faveur de la prudence que doit montrer tout expérimentateur dans l'appréciation des résultats d'un traitement et montre l'importance des contrôles répétés et ceci pendant *très longtemps*. Ce seul échec à côté de nombreuses guérisons ne retire rien à la valeur thérapeutique du TWSb, médicament dont la remarquable activité sur l'élimination des œufs doit retenir l'attention des épidémiologistes, mais il démontre une fois de plus, comme nous l'avons déjà écrit (*Bull. Soc. Path. Exot.*, 1956-49-6-1220), que, si nous disposons de médicaments utilisables pour l'épidémiologiste capables de réduire l'endémie bilharzienne en interrompant momentanément l'élimination des œufs, il reste encore à découvrir le produit qui permettra en une cure d'obtenir une guérison définitive de la maladie objectif que vise le médecin qui traite un malade atteint de bilharziose et soustrait à la possibilité de nouvelles infestations.

DR. JAMAL A. AZZAWI (Iraq) — In Basrha area, the problem of bilharzia is very complicated and interesting. There is definitely a demarcation line of endemicity.

The personnel of W.H.O. Bilharzia Control, Projec — Endemic Diseases Institute, Baghdad, Iraq, has made a urine survey among children living around the 9 main canals of the Basrha area in

June 1958. The results indicate a high human infection rate along the 4 northern canals of the city of Basrha, the infection rate ranging from 86-20 percent. In the fifth canal the infection rate was 2 percent, in the sixth, seventh, eighth and ninth canals there was no infection.

A snail survey was done simultaneously in the area and, surprisingly enough, *Bulinus truncatus*, which is the only intermediate host known in Iraq, was not detected in any of the 9 canals.

At the same time, environmental factors were studied in the water courses, such as velocity, turbidity, pH value, and salinity of the water. All these factors showed no significant difference in the canals examined.

Dr. Watson has reported that the absence of human infection south of Basrha is due to high salinity of water in Shatt-al-Arab resulting from an influx of sea water during high tide, which makes the water unsuitable for snail life.

In fact, the sea water does not mix with Shatt-al-Arab water during high tide, as there is a demarcation zone which does not exceed a distance of 5 km north of Fao Port on the Arab Gulf (Persian Gulf).

In our survey, in 1958, we obtained reliable information from the Hydrological Department of Basrha Port; according to their repeated water analysis, the estimated salinity was as follows:

In Lake Hammar, 25 km north of Basrha, salinity was estimated at 2,000 ppm. In Garmat Ali, a canal which takes water from this lake to the Shatt-al-Arab, 6 km north of Basrha, the salinity was estimated to be about 1,200 ppm. Both these figures are below the tolerated level of salinity of *Bulinus truncatus*, which is above 4,000 ppm. (Wright, 1958).

In the canals of the city of Basrha and its vicinity, salinity was hanging between 700 and 800 ppm.

It was concluded that salinity of water north of the city of Basrha is higher than the salinity in and south of Basrha, but the human infection canals of Basrha and up north, from Basrha to Qurna town, while the snail vectors could not be detected in the northern canals of Basrha and were only found in small quantities and irregularly between Basrha and Qurna town.

So the salinity of the water is not a factor that explains the absence of human infection south of Basrha, as the tolerated level of

salinity of *Bulinus truncatus* is much higher than the salinity levels found in the water courses of Basrha area and down south to Abu Khasib, 30 km south to Basrha.

This finding leads us to think of the following: *First* a more extensive survey for the snail intermediate host should be done in Basrha area in various seasons of the year, if proved negative again the *second* possibility is a presence of another intermediate host or a *third* that the human infection in that area is due to *Schistosoma bovis*. We are working on this problem at the present time and hope to get a satisfactory result.

I have asked Dr. Gönnert whether the Molluscicide Bayer 73 kills the eggs of the snail, and the answer was yes.

RUDOLF GÖNNERT (Germany) — *Answer to the question of Dr. Szumlewicz*: In reply to the question about the residual effect which has been raised by Dr. Szumlewicz, I cannot furnish as yet any data based on systematic field trials. Such trials still have to be carried out. Tested in the aquarium however, and in comparison to pentachlorophenole, Molluscicide Bayer 73 proved to be of superior residual effectiveness. *Answers to the questions of Dr. Buttner*: As to the price, no definite statements can be made at this point, which is natural. But Bayer 73 does have biological effects superior to those of other molluscicides. As a result of this, smaller amounts of the substance will be used in the field, which in turn will cut the costs of packing, transporting, etc. We therefore are convinced that Bayer 73 will be competitive.

Dr. Buttner's second question, concerning the *in situ* effect in running water, can only be answered once we have the results of the field trials.

SUBSECTION 2

FILARIASIS

1ST SESSION: *Onchocerciasis*

Friday, September 5th—4:30 to 18:00 p.m. Room C

The meeting was opened by the Chairman, Prof. Dr. Henri Galliard. The Rapporteur was Dr. W. Kershaw and Dr. Mário M. Araújo Abreu acted as Secretary.

The following papers were presented and discussed.

PATHOGENESIS OF EYE-LESIONS IN ONCHOCERCIASIS ¹

W. E. KERSHAW

Liverpool School of Tropical Medicine

The eye-lesions which have been associated with *Onchocerca volvulus* may involve the anterior segment of the eye, producing lesions of the cornea, the iris and the anterior chamber; or the posterior segment, producing optic atrophy and chorio-retinal lesions.

ANTERIOR EYE-LESIONS

The anterior eye-lesions are related to the intensity of the infection in the head and neck.

The distribution and concentration of the microfilariae of *O. volvulus* in the skin in the African form of infection have been shown to have a definite pattern which is related to the skin lesions and

¹ This paper is, in substance, a modification of a contribution to a Symposium on Onchocerciasis held at the Royal Society of Tropical Medicine & Hygiene, London, in January, 1958, and published in the *Transactions* of that Society, vol. 50, p. 122. Acknowledgement is made to the Editor of the *Transactions*, and to the Editor of the *British Medical Journal*, for permission to reproduce the figures.

to eye changes in the anterior segment of the eye (Kershaw, Duke and Budden, 1954; Kershaw, Jamison, Nugent and Duke, 1956).

In an early or lightly infected case, the microfilariae may be found only in the same anatomical region as the nodule.

As the case increases in intensity, there is a progressive rise in the concentration in the legs and lower trunk with a spread of microfilariae to the shoulders, face, conjunctiva and to the anterior chamber of the eye.

Microfilariae can thus reach the anterior chamber of the eye by two mechanisms:

(1) An increase in intensity of infection from prolonged exposure, resulting in a rise in the level of the microfilariae to the head and neck—the usual pattern in West Africa.

(2) A local increase in the neighbourhood of a nodule on the head—probably the usual pattern in America, and occurring there at an earlier stage of the infection than in the pattern of the infection in Africa—or as an incidental occurrence during the course of an infection of the usual pattern in Africa.

(These two patterns of infection, the African and the American—though this form awaits accurate confirmation—correspond to the differences in the biting habits of the African and American vectors.)

The local intensity of infection in the shoulders, neck and head in both forms of the infection can be measured directly and conveniently by a quantitative examination of a skin snip taken from the shoulder.

That the anterior eye-lesions are related to the intensity of infection in head and neck is shown by Figs. 1, 2, and 3, where the pattern of the infection in Africa is illustrated, and in Fig. 4, in which the relation between anterior eye-lesions and the intensity of infection at the shoulder is shown by frequency distribution diagrams.

POSTERIOR EYE-LESIONS

The posterior eye-lesions are not related to the intensity of infection in the head, neck, or elsewhere.

No relation was found comparable to that illustrated in Figs. 1, 2, and 3; nor in Fig. 4, where the frequency distribution diagrams of

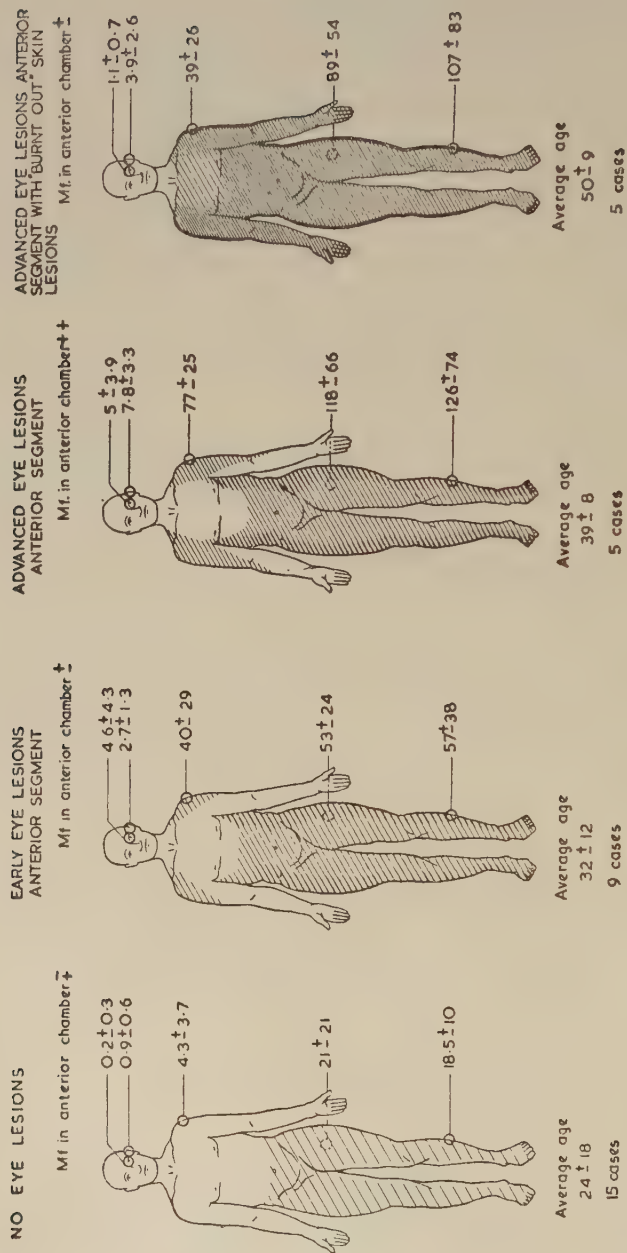


Fig. 1 — Showing the relation of the distribution and concentration of the microfilariae of *O. volutus* to the skin lesions and to the changes in the anterior segment of the eye in the inhabitants of the village of Gbulki. The concentration is given as the number of microfilariae per milligramme of skin over the calf, buttocks, deltoid region, and the outer canthus of the eye, and of the conjunctiva. The areas with marked skin changes are shaded. The mean number of nodules found in the individuals in the four groups is 1.9 ± 1.3 , 4.2 ± 3.2 , 5.4 ± 2.7 and 4.4 ± 2.3 . Most of the nodules are on the pelvis, or the legs, and none is on the head

the number of subjects with posterior eye-lesions, plotted against the intensity of infections at the shoulder, is the same as those with no eye-lesions.

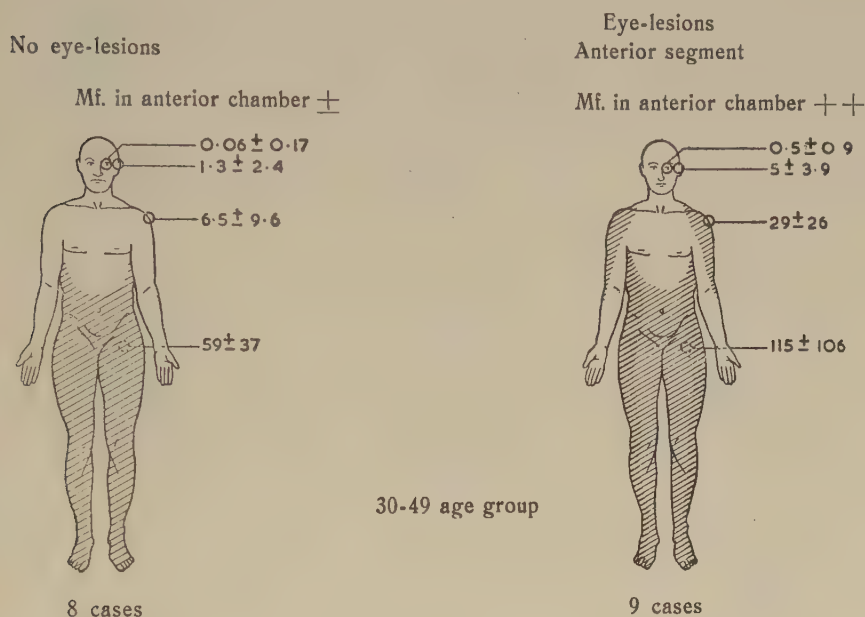


Fig. 2 — Comparing the distribution and concentration of the microfilariae of *O. volvulus* in those with no lesions of the eye and in those with lesions of the anterior segment in the age-group 30-49 of the inhabitants of the village of Bonjongo. The concentration is given as the number of microfilariae per milligramme of skin over the buttock, deltoid, and the outer canthus of the eye, and of the conjunctiva. The areas with marked skin changes are shaded. The mean number of nodules found in the individuals in this group is 4.6 ± 2.1 and 9.3 ± 4.9 . The intensity of infection is higher and the spread of microfilariae more advanced in those with anterior segment lesions than in those whose eyes are not involved, showing that the resulting intensity of infection, and not merely age and duration of exposure, is the causal factor

As the posterior eye-lesions are not related to the intensity of infection, they may:

- (i) not be related to infection at all, but due to a condition which is presented as an accidental association,
- (ii) be related to infection in a qualitative sense,

(iii) be related to another basic condition which may be influenced by infection in either a qualitative or, less probably, in a quantitative sense.

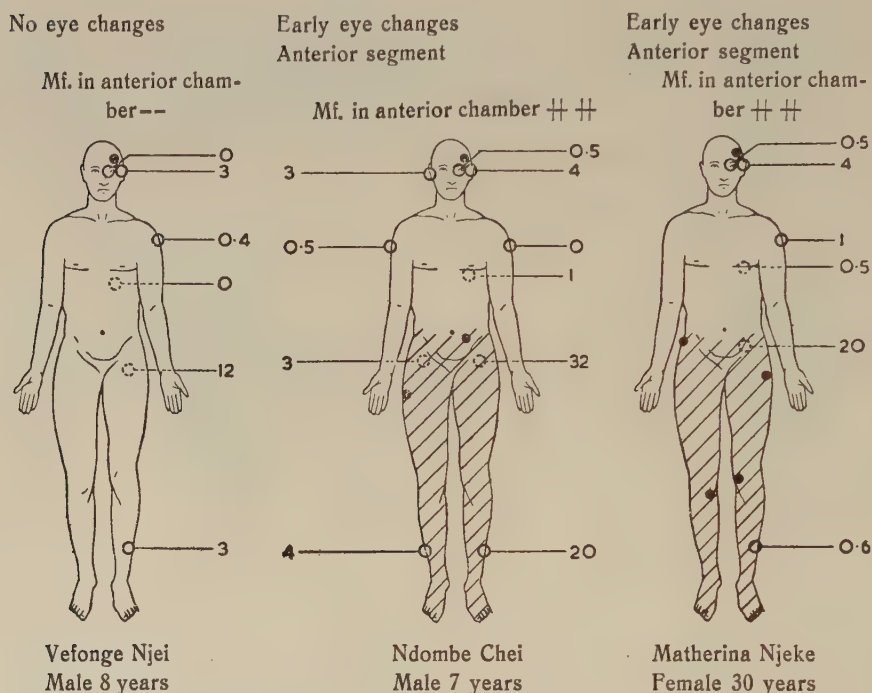


Fig. 3 — Showing the relation of the distribution and concentration of the microfilariae of *O. volvulus* to the skin lesions and to the changes in the anterior segment of the eye in the only 3 individuals out of 200 examined by multiple skin snips in the north of Nigeria, in the Cameroons, who had nodules on the head. In the first child the concentration is beginning to rise in the buttocks, but there is in addition a local rise in the outer canthus of the eye and in the shoulder near the nodule on the head. No eye changes have developed. In the second child and in the adult woman, the local rise in the concentration in the head and neck with early eye changes in the anterior segment have occurred as an incidental in cases of infection of the usual pattern occurring in Nigeria

As my colleague Choyce (1958) has pointed out, choroido-retinal lesions, established as being of a genetic nature, occur in temperate climates, and are indistinguishable from those found at Bonjongo,

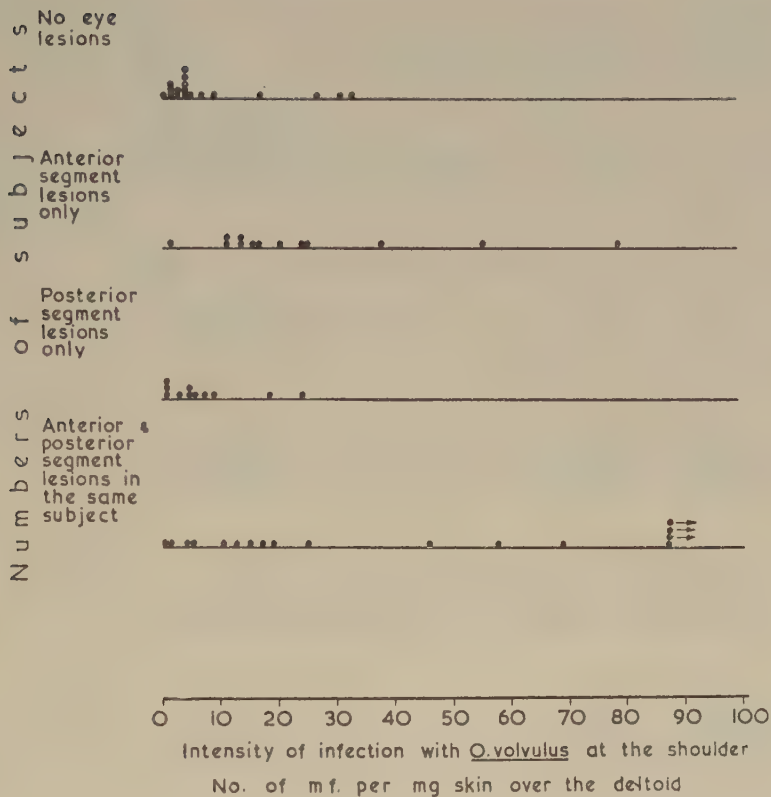


Fig. 4 — Diagrams showing the relation between the lesions of the anterior and posterior segments of the eye and the intensity of infection with *O. volvulus*, constructed from data obtained during surveys in 1954 and 1957 at the village of Bonjongo, in the British Cameroons. The frequency distribution diagrams show the number of subjects with (a) no eye lesions, (b) anterior segment lesions only, (c) posterior segment lesions only, and (d) anterior and posterior segment lesions in the same subject, plotted against the intensity of infection at the shoulder (number of microfilariae per milligramme of skin over the deltoid). The data have been limited to those subjects over 30 years of age, since those who are younger are more migrant. The results of both surveys, one carried out in 1954, in which the ophthalmological examinations were made by Dr. F. H. Budden, the other in 1957 with Mr. D. P. Choyce, gave, separately, similar results

in the Cameroons, and similar to those described by other ophthalmologists working in West Africa. This alternative constitutional explanation for posterior eye-lesions seen in those infected with

O. volvulus is compatible with (i) and (iii) and should therefore be investigated by appropriate genetic and epidemiological methods.

Though posterior eye-lesions have in the past been traditionally associated with infection with *O. volvulus*, most of the surveys for blindness have been carried out in areas where onchocerciasis is known to exist, and less attention has been paid to other areas. Much more accurate information is required on the detailed ophthalmological aspects and on the intensity of the infection in different areas in Africa and in America.

Geographical coincidence is not always a reliable criterion in the attribution of pathological effects to particular parasites, for the cause of African sleeping sickness was at one time seriously considered to be the filaria then called *Filaria perstans*, a belief then supported by apparently sound clinical and epidemiological evidence.

ANTERIOR AND POSTERIOR EYE-LESIONS AND THEIR ASSOCIATED DISABILITIES

In our experience, limited to certain parts of West Africa, the anterior eye-lesions cause relatively mild diminution of visual acuity and but rarely economic blindness (Fig. 5) whereas the posterior lesions are associated with severe diminution of visual acuity and with economic blindness.

It now becomes necessary to determine how far this clear difference in the disabilities caused by the two types of lesions is true of other areas in West Africa, where the intensity of infection may differ, such as in the northern territories of Ghana, where, in places, the intensity of infection is probably very high, and to compare it with that in Sierra Leone, where it is probably low; and in America, where it would be expected that the level of intensity in the head and neck would be higher than the corresponding level in the Africans exposed to a similar risk of infection for the same length of time.

Because of the economic blindness due to the posterior eye-lesions, it is of immediate practical importance to determine precisely the cause of these lesions, and to define closely whether they are related to

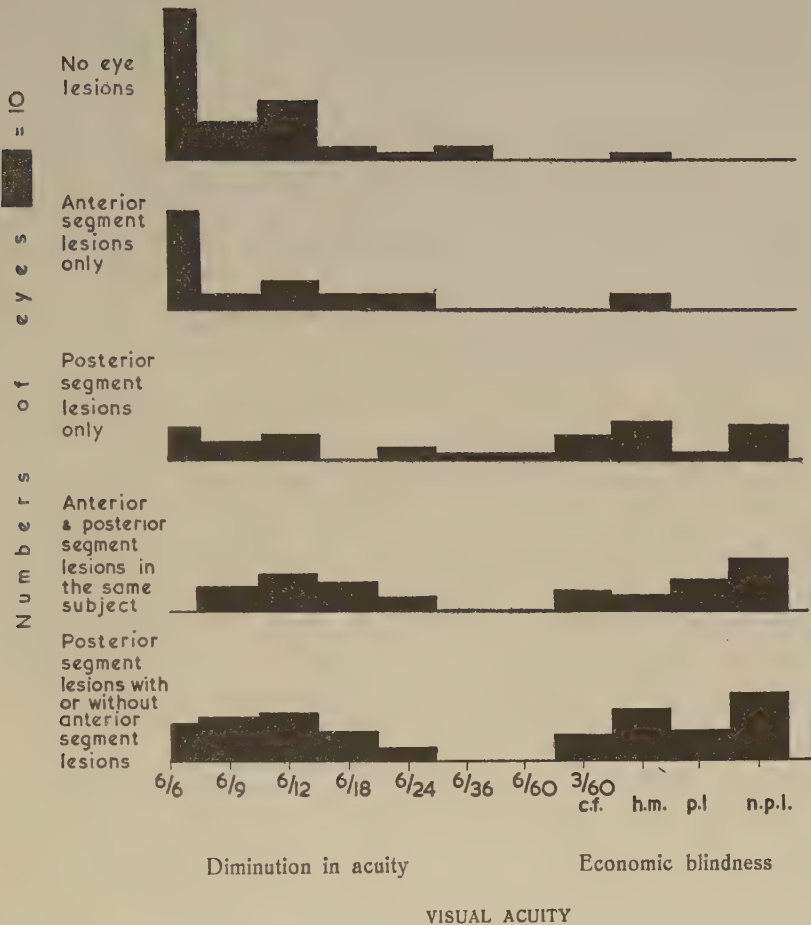


Fig. 5 — Diagrams showing the relation between the lesions of the anterior and posterior segments of the eye and their associated disabilities constructed from data obtained during surveys in 1954 and 1957 at the village of Bonjongo, in the British Cameroons. The frequency distribution diagrams show the number of eyes with, (a) no eye-lesions, (b) anterior segment lesions only, (c) posterior segment lesions only, (d) anterior and posterior segment lesions in the same subject, and (e) posterior segment lesions with or without anterior segment lesions, plotted against visual acuity (c.f. = count fingers; h.m. = hand movements; p.l. = perception of light; n.p.l. = no perception of light). The results of both surveys, one carried out in 1954, in which the ophthalmological examinations were made by Dr. F. H. Budden, the other in 1957, with Mr. D. P. Choyce, gave, separately, similar results

infection with *O. volvulus* or not; should they merely be accidentally associated with infection, they are unlikely to be influenced by control measures.

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TRANSMISIÓN DE LA ONCOCERCOSIS

F. DÍAZ

Guatemala está situada en América Central y su superficie es de 113.000 km², de los que 1.877 sufren de endemia oncocercosa.

Fue allí donde el Dr. Rodolfo Robles estableció en 1915 que el simúlido es el vector de la parasitosis y que esta infestación produce las lesiones oculares que pueden llegar hasta la ceguera y las de la piel que nuestros labriegos llaman *erisipela de la costa*.

Espigaré rápidamente en el fecundo campo de la historia de la oncocercosis:

- En 1795 Schönbauer descubre que el *S.* se cría en el agua corriente;
- En 1875 O'Neill encuentra la microfilaria en la piel de quienes sufren de *craw-craw*;
- En 1893 Leuckart descubre la *Filaria volvulus* en un nódulo, y la describe;
- En 1915 Robles descubre y magistralmente describe la oncocercosis americana;
- En 1916 Robles aísla entera la hembra de la *oncocerca volvulus* haciendo digerir por un perro el oncocercoma durante 5 horas;
- En 1926 Blacklock comprueba que, como Robles lo previó, son los *S.* los vectores de la *volvulus*, e incrimina al *damnosum*; Garnham y McMahon ensayan el DDT como larvicida;
- En 1930 Hoffmann describe la evolución de la *oncocerca* hasta la forma de salchicha en el cuerpo del *S. callidum* al que juzga el único seguro vector; Ochoterena ve la mf. en el ojo muerto, y Torroella la ve en el ojo vivo;

- En 1932 Hissette encuentra en Africa las oncoftalmopatías en América denunciadas por Robles en 1915 y descritas por Pacheco Luna en 1918, descripción que, al decir de Hissette, «sigue siendo verdad»;
- En 1933 Díaz sospecha que existen oncocercomas inaccesibles a la palpación;
- En 1934 Giaquinto encuentra mf. en el nervio óptico;
- En 1943 Díaz indica cual es, en su concepto, la evolución de la oncocerca en el organismo humano;
- En 1944 van Hoff y Wanson ensayan la Germanina y con ella matan la oncocerca adulta;
- En 1948 Mazzotti y Hewitt encuentran que el poder filaricida del Hetrazan también se manifiesta en la volvulosis.

La total metamorfosis del *S.* requiere de 12 a 54 días, según sean las condiciones ecológicas y la especie de que se trate; en Guatemala la del ochraceum se efectúa entre 12 y 31 días.

En algunas regiones los imagos pueden encontrarse relativamente lejos de sus criaderos, por lo que éstos no deben buscarse únicamente donde se encuentran adultos.

Se ha llegado a ver *S. damnosum* a 25 millas (40 km) de sus criaderos; el ochraceum y el metallicum no se han encontrado a más de 15 km y, si están parasitados, a no más de 5 km.

Según Giaquinto (13), la antropofilia del *S. americano* es así: ochraceum 98 por ciento; metallicum 13 por ciento; callidum 3 por ciento.

Hoffmann (14) y Dalmat (correspondencia personal) piensan que en América el principal vector es el callidum, del que Vargas logró mantener vivas 3 hembras durante 21 días, mientras Giaquinto (13) y de León (9) dicen lo es el ochraceum.

Se piensa que éste es el principal vector, 1.º por sus hábitos antropófilos; 2.º porque prefiere picar en la mitad superior del cuerpo; 3.º por el tiempo de succión de sangre; 4.º porque su geografía es la más coincidente con la de la endemia, sin por eso superponerse.

Aún se ignora porqué no hay oncercosis en regiones en que existe el *S. transmisor*. No es porque falte el eslabón humano, como pensaba Strong, pues Díaz en 1937 vio conviviendo *S.* vectores y

oncercosos foráneos con larga residencia, y ni un solo caso autóctono: observación semejante hizo de León.

La conducta de los *S.* cambia con las regiones y las condiciones ecológicas. La misma especie tiene hábitos distintos en comarcas distintas; así el *neavei* pica la cabeza en Kinesi, Uganda, el tronco en el Nyanza meridional y los pies y las piernas en el Nyanza septentrional.

Los logros de Garnham, McMahon y colaboradores en Kenya nos enseñan como combatir la oncocercosis en América. Las condiciones ambientales son distintas en uno y otro continente, pero en esa diferencia, así como hay factores que aumentan las dificultades de la labor, como la gran multiplicidad de cursos de agua, hay otros: área menor, menos difusión de los poblados, mejores vías de comunicación, colaboración de los terratenientes, que la hacen más fácil.

El trabajo larvicida contra el *S.* debe ser intenso, simultáneo y bien organizado, para lo cual es indispensable cartografiar con auxilio de la aerofotografía y conocer en todas sus características cada una de las corrientes de las zonas contaminadas y de las regiones aledañas; contar con el dinero necesario y, principalmente, con numeroso personal moral y técnicamente capacitado para hacer esa guerra relámpago que debe durar no menos de 3 meses: la erradicación del vector en la zona de endemia y hasta 50 km de su periferia indicará la suspensión de la labor.

Para triunfar son necesarios los siguientes factores: *a*) aplicación del insecticida en las corrientes siguiendo un orden apropiado para evitar la reinfestación originada en los afluentes: una sola corriente no tratada causaría el fracaso total; *b*) como se dijo, el personal debe ser capacitado y moralmente responsable; *c*) contar, en lo posible, con buenas vías de comunicación y con vehículos en buen estado y en suficiente número; *d*) perfecto conocimiento de la ecología local.

En Guatemala, Dalmat (8) durante 3 meses experimentó aplicando el insecticida cada 21 días a 1.500 corrientes ubicadas en una área de 80 millas cuadradas (207 km²). El 85 por ciento de ellas tenía menos de 100 galones (378 l) por minuto. Regularmente bastó una aplicación de DDT de 0,1 ppm en 3 minutos para dejarlos sin larvas. En arroyos largos y en ríos de 5.000 galones (18.927 l) por minuto, el mismo tratamiento escalonado y en distintos puntos de

su curso. En ríos mayores y de difícil acceso se aplicó el DDT 2 ppm durante 3 minutos o 0,1 ppm 60 minutos: éste llegó a mayores distancias y en los 3 meses siguientes disminuyeron los imagos.

La dosificación más usada de DDT es 1 ppm durante 30 minutos. Y, para evitar la reinfestación, cada aplicación global no se hará en más de 10 días.

Debe recordarse que las ninfas, en el aire húmedo, sobreviven largo tiempo.

La solución madre de DDT se hará al 1 por ciento en acetona, agua o alcohol.

La vida de la volvulus transcurre en 3 etapas sucesivas. La 1.^a como huesped de un animal de temperatura constante, en forma de huevo y de larva en su primer estadio; la 2.^a en el tórax y los tubos de Malpighi del *S.* (9) en que sufre metamorfosis para las que son necesarios de 4 a 7 días (1, 2, 7); la 3.^a en el hospedero definitivo en que continúa su transformación hasta hacerse adulto y cerrar el ciclo.

Es la 3.^a etapa cuyo calendario creo haber encontrado, fijando unos 8 meses entre el piquete infestante y la formación del oncocercoma, y de 5 a 7 para que la hembra sea fecundada y la mf. llegue a la piel. Nunca he encontrado formas evolutivas en el nódulo, por lo que pienso que la oncocerca se enquistas cuando ha adquirido su madurez sexual. Calculo (10) en unos 8 meses la vida de la mf. cutánea, y en más o menos 20 años (11) la del gusano adulto, cuya muerte causa la absorción, calcificación o supuración del quiste. Siendo la oncocerca un animal sexual, la víctima necesita ser picada por lo menos 2 veces para ser portadora de mf.

Rara vez se encuentran metacíclicas en la proboscis porque la migración al labium se efectúa mientras el *S.* pica (8) y, como no se sabe en que momento de la succión se desplaza, pienso que vector y parásito deben inmovilizarse (con cloruro de etilo, por ejemplo), a intervalos de 15 a 30 segundos, para poder encontrar con más frecuencia la larva infestante, y hasta para sorprenderla cuando esté penetrando la piel.

Que yo sepa únicamente han visto metacíclicas en la proboscis del *S.*, Strong, Nettel, Manson-Bahr, Blacklock, Rochet y quien esto escribe. En cambio Hoffmann y de León, a pesar de numerosas y muy concienzudas disecciones, no la han hallado una sola vez.

En 1928, cuando nada se sabía de la biología de la mf., Villalobos (15) piensa que está en las hojas de las plantas y que su contacto con la piel permite la infestación.

Sabido es que las B. en las oncodermatosis son menos fértiles que en la piel aparentemente normal, y pienso que esta exacerbación semiológica se debe a la muerte y desintegración de los helmintos, y no a su brusca y exagerada producción, como se cree corrientemente.

Enorme interés epidemiológico tiene la posibilidad afirmada por varios investigadores (20, 22) de que animales, especialmente bovinos, sean reservorio de la volvulus. Rodger (18) va aún más lejos: piensa que volvulus y gibsoni son sinónimos y que su única diferencia morfológica es el tamaño de la mf., diferencia que explica por la mayor talla del receptor bovino, como la *Fasciola gigantica* que se achica cuando pasa de la vaca al conejo (17).

Sharp (19), van den Bergh (21), Díaz y un estudiante guatemalteco de medicina cuyo nombre ignoro, han encontrado oncocercas libres en el cuerpo del hombre.

Respecto a los oncocercomas situados en lugares donde la palpación es imposible y que fueron hallados porque había otros superficiales cercanos y se buscaron después de la extirpación de éstos, Díaz (11) ha encontrado los siguientes: 3 en la cara anterior de la escápula, 1 en la cara pleural de una costilla, 1 en la cara anterior de la articulación sacro-coccígea y 1 bajo los glúteos, cerca de la articulación sacro-iliaca.

Si se practicaran autopsias pensando encontrar, no sólo oncocercas libres sino también oncocercomas profundos, los que, en mi concepto, deben buscarse cerca del esqueleto, creo se resolvería el porqué de las BP sin oncocercoma aparente, si es que se considera aún no resuelto con los hallazgos de Díaz.

Robles (16) vio 4 cráneos completamente perforados por oncocercomas, Calderón (5) vio 3 y Díaz (12) también 3. Uno de ellos cuya es la fotografía que me permito presentar y que fue de una anciana a quien extraje el nódulo en marzo de 1938 y falleció en 1939: exhumé la pieza en 1945.

La B. es un medio diagnóstico que sistemáticamente debe hacerse a quienes no se encuentra oncocercoma y hasta reforzarse con el

xenodiagnóstico, para repetir la palpación a quienes tengan BP, para someter a tratamiento químico adecuado a quienes con BP no se encuentren nódulos, o simplemente con fines estadísticos. En 1950 (4) al 50,53 por ciento de personas con BP no se encontraron oncocercomas en mi patria.



Fig. 1 — Perforación total del cráneo, por oncocercoma

Generalmente se aconseja que, si la biopsia es negativa, se hagan hasta 4 más (6). No estoy de acuerdo con este procedimiento, que aumenta el trabajo y lesiona tantas veces al paciente. Por lo regular no hago más de 2 y, si son negativas, las pongo en una cámara húmeda improvisada y las reexamino al día siguiente: con gran frecuencia las encuentro positivas. Tampoco dislacero la B.: la espera hace salir la mf. con mayor facilidad, si el ambiente es apropiado.

Es indudable que hay individuos inmunes a la oncocercosis como Robles suponía (16). Sólo así se explica que hayan permanecido expuestos al contagio toda su vida, a veces de muchos años, y continúen indemnes. En cambio hay otros que se reinfestan constante-

mente. También es de notar la propensión de algunas familias a las complicaciones oculares.

Blacklock (3) y de León intentaron infestar con metacíclicas el 1.º dos monos y el 2.º un voluntario; Robles (16), Strong (20), Calderón (5), de León y yo (11) hemos colocado oncocercomas bajo la piel de monos, perros, conejos y cobayos. A los conejos y a los cobayos con que experimenté se les hicieron repetidas biopsias, se les buscaron microfilarias en la sangre, se les tomaron fórmulas leucocitarias y se les observó hasta que los nódulos se absorvieron: el resultado de estas experiencias fue negativo.

La oncocercosis no se extiende geográficamente sino por excepción. Los focos descubiertos recientemente en Guatemala existían desde tiempo inmemorial, excepto uno pequeño en el oriente del país, cuyo origen pude establecer a plenitud. Hoffmann y Vargas (23) encontraron en Chiapas, México, focos iniciados, lo más, u año antes.

La volvulosis en Africa ha despoblado regiones muy fértiles cuyos habitantes huyen por temor a contagiarse y cegarse. Las vecindades de los ríos, las tierras ubérrimas, son las más temidas. La gente tala las montañas, pues sabe que la selva protege el simúlido.

En Guatemala y en México esta parasitosis existe en las regiones cafetaleras, y el café es un producto de exportación que contribuye a mejorar nuestra balanza comercial, así que la desvalorización del capital humano repercute sensiblemente en nuestra economía.

Ojalá que esta justa científica intensifique aún más la lucha contra este terrible flagelo.

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ENFERMEDAD DE ROBLES

(ESTADO ACTUAL DE LA ONCOCERCOSIS EN GUATEMALA)

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Las características médico-sociales de la enfermedad de Robles, señaladas desde la comunicación magistral de su descubridor en el continente americano en 1915, confirmadas posteriormente por numerosos trabajos y puestas de relieve recientemente en la Conferencia de Léopoldville, en 1954, nos han estimulado a traer este tema a la Subsección de Filariasis del Sexto Congreso Internacional de Medicina Tropical, máxime que en el momento actual, aún constituye un serio problema de salud pública, no sólo en Guatemala, sino en otros países de América y en más de la mitad del continente africano. Las recomendaciones que al respecto pudieran proponerse serán de suma importancia en la lucha que se tiene entablada contra dicha enfermedad.

Expondremos este trabajo en la siguiente forma: I. — Antecedentes; II. — Estado actual de la enfermedad de Robles (oncocercosis) en Guatemala; III. — Conclusiones.

I. — ANTECEDENTES

Es de todos conocido que la descripción del parásito *Onchocerca volvulus* fué hecha por el notable zoólogo Leuckart en 1893, en los helmintos que encontró en los tumores que le fueron remitidos

por un médico-misionero alemán, quién los extirpó a dos negros en la Costa de Oro (1).

Prout, en 1901, observó dos casos en Sierra Leona y el Prof. Brumpt, en su expedición de 1904, encontró nódulos oncocercósicos en nativos del Africa Central.

Según el Prof. A. J. Rodhain (2), el estudio publicado en 1913 por el médico militar francés Ouzilleau llamó la atención en Africa sobre la significación para el hombre del parasitismo debido a la *Onchocerca volvulus*. Residiendo en el territorio Oubangui-Bomu, reveló la gran frecuencia de portadores de nódulos verminosos, correspondiendo con la elefantiasis del escroto, adeno-linfoceles y ciertas alteraciones querato y esclerodérmicas. El Prof. Rodhain confirmó en 1915 las observaciones de Ouzilleau en el territorio del Uélé, en la vecindad del Congo Francés.

Al final de su discurso, el Presidente de la Conferencia de Léopoldville dice textualmente: «Le rôle du parasitisme à *Onchocerca volvulus* dans l'étiologie de l'éléphantiasis des organes génitaux externes et du faux adéno-lymphocele demeure un sujet de controverse.»

Tácitamente el Prof. Rodhain reconoce que en las observaciones de Ouzilleau no puede descartarse la coincidencia de lesiones ajenas a la *Onchocerca volvulus*.

Sin pretender restar mérito alguno a los precursores del estudio de la oncocercosis, podemos afirmar categóricamente (y así lo han hecho todos los autores guatemaltecos que se han ocupado del tema, opinión compartida por numerosos investigadores extranjeros) que las cuidadosas observaciones de Robles, efectuadas de 1915 a 1917, tuvieron como resultado dejar un cuadro completo de la enfermedad: describió el parásito, trazó con maestría (3) las formas clínicas, señalando por primera vez las lesiones oculares, y atribuyó la transmisión de la *Onchocerca* a insectos del género *Simulium* (hipótesis comprobada por Blacklock en Sierra Leona, en 1926). Así mismo Robles instituyó las bases del tratamiento quirúrgico, que con pocas variantes se ha venido siguiendo desde entonces.

También es de todos sabido que las lesiones oculares fueron descritas por el notable oftalmólogo guatemalteco Dr. Rafael Pacheco Luna, en 1918, teniendo que esperar hasta «el año 1931 para que el médico oculista belga Hissette (2) estableciera de una manera defi-

nitiva que las lesiones oculares se encontraban en portadores de nódulos verminosos en el Congo Belga».

El resultado de las observaciones y experiencias de Robles fué dado a conocer en una memorable conferencia en el seno de la sociedad La Juventud Médica (4), el 4 de marzo de 1917, y posteriormente en la Sociedad de Patología Exótica, en París, en julio de 1919 (5).

En un trabajo anterior (6) presentamos la bibliografía guatemalense de la enfermedad de Robles; dijimos entonces: «cronológicamente se puede considerar dos etapas: la primera, consecutiva al descubrimiento efectuado por el Dr. Robles, seguida de los trabajos de Pacheco Luna, Calderón, Estévez, Morales, Fletes Sáenz, Guerrero y Azurdia; separada por un espacio de cinco años, la segunda etapa de la bibliografía guatemalense sobre oncocercosis se continuó con la Tesis de Alvarez, en 1929, siguiéndole los trabajos realizados en la Dirección General de Sanidad Pública, por Muñoz Ochoa, Estévez, Marroquín G., Díaz y de León; comprendimos dentro de esos trabajos los realizados por Mira y Jacobsthal, concluyendo el referido reporte con la tesis de García Valle, publicada en 1947. Aunque probablemente hayamos omitido algunos, logramos reunir 59 referencias bibliográficas de autores guatemalenses referentes al tema, habiendo sido reproducidos algunos de esos trabajos en el volumen *Oncocercosis (Enfermedad de Robles)*, que la Asociación de Oftalmología de Guatemala, con el apoyo de la Facultad de Ciencias Médicas y de la Universidad de San Carlos, presentó como homenaje al Tercer Congreso Panamericano de Oftalmología, celebrado en La Habana, en enero de 1948.»

En los últimos diez años han sido realmente pocos los trabajos nacionales que se han ocupado de la enfermedad de Robles, no siendo sino las comunicaciones de Pacheco Luna, Peñalver, de León, Díaz y Bernhard, éstos tres últimos con el autor, que tomaron parte en la mesa redonda de oncocercosis, auspiciada por el VII Congreso Nacional de Medicina de Guatemala, diciembre de 1957.

Debemos hacer especial mención de los trabajos efectuados por la expedición de la Universidad de Harvard, integrada por Strong, Sandground, Bequaert y Ochoa, efectuados en 1932 y publicados en 1934 en un interesante libro: *Onchocerciasis, with special reference of the Central American form of the disease* (7). No es

nuestra intención, ni el espacio disponible lo permitiría, el hacer un resumen de los capítulos de tan importante monografía, ya que es conocida de la mayoría de autores que se ocupan del estudio de la enfermedad de Robles.

De gran importancia son así mismo los trabajos efectuados por Burch, Gibson, Dalmat y Lea, quienes, auspiciados por la Oficina Sanitaria Panamericana y el Instituto Nacional de Salud Pública de los Estados Unidos, llevaron a cabo un extenso programa durante cerca de 5 años, estableciendo un Centro de Investigaciones en el Municipio de San Pedro Yepocapa, del departamento de Chimaltenango. Pesquisas exhaustivas, de las cuales fuimos testigos, en quimioterapéutica, parasitología, entomología y control por el empleo de insecticidas, en uno de los estudios más sistemáticos y continuados que probablemente sobre la enfermedad de Robles se han efectuado hasta la fecha. Son numerosas las publicaciones de dichos autores, en las que relatan los resultados obtenidos; de ellas está ya condensado en un volumen el trabajo de Dalmat: *The Black flies (Diptera, Simuliidae) of Guatemala and their role as vectors of Onchocerciasis* (8).

Los trabajos fundamentales de Robles, Pacheco Luna y demás autores guatemaltecos estimularon la investigación en la oncocercosis: en 1920-21, Montpellier y Laigret (2) comprobaron la presencia de microfilarias en la dermis de soldados negros africanos atacados de lesiones pruriginosas, lo que ya había sido sospechado por Robles, cuando dijo: «La linfangitis en estado agudo se termina con agrietamiento de la piel de donde mana un abundante líquido seroso y donde creemos que deben existir los gérmenes.» (4). Robles tiene así mismo la prioridad en la hipótesis de la transmisión por los simúlidos y en haber señalado las lesiones oculares (descritas por Pacheco Luna). Quedan también, como contribuciones importantes en el conocimiento de la enfermedad de Robles, las descripciones histopatológicas de Estévez (9), los ensayos quimioterapéuticos por inyecciones intranodulares — Díaz, (10) — y la identificación de nuevas especies de simúlidos por de León (11), para no citar sino a las de mayor importancia.

En México, Benítez Soto y Sámano Bishop (12) refieren que en 1920 Bustamante indicó la posible existencia de la oncocercosis; Villalobos encontró, algunos años más tarde, el foco oncocercoso de

Chiapas, y Larumbe, poco después, el de Oaxaca. Torroella, Ocho-terena, Mazzotti, Figueroa-Ortiz, Nettel, Hoffmann, Vargas, Puig-Solanes, Ruiz Reyes y otros investigadores mexicanos han contribuido eficazmente al mejor conocimiento de la enfermedad de Robles.

Según Gradwohl (13), el primer caso de oncocercosis fué encontrado en Venezuela por Potenza en 1948. Posteriormente, el mismo autor, en colaboración con González, Vogselsang, Anduze y Torres, publicaron el resultado de sus estudios, determinando las características epidemiológicas de la enfermedad, localizándola en los estados de Monagas y Sucre, con una infestación global de 24.6 por ciento, comprobando la presencia de simúlidos transmisores en las zonas afectadas.

Martínez Báez (14) indica que la enfermedad de Robles ha sido señalada en la Guyana Inglesa en 1955.

En la actualidad el número de trabajos sobre oncocercosis es muy considerable; ya en 1950, la Oficina Sanitaria Panamericana publicó la bibliografía, incluyendo estudios seleccionados hasta junio de 1945 (15), reuniendo en él 1615 fichas y referencias. Sería conveniente que a un complemento a tan importante obra se agregara los trabajos que sobre enfermedad de Robles se ha publicado a partir de junio de 1945.

II. — ESTADO ACTUAL DE LA ENFERMEDAD DE ROBLES (ONCOCERCOSIS) EN GUATEMALA¹

A. — DISTRIBUCIÓN GEOGRÁFICA

Las áreas de distribución permanecen estacionarias desde hace 10 años, es decir, prácticamente están idénticas a las referidas el año de 1948. Comprenden:

a) *Zona N.º 1* — Abarca regiones de los departamentos de Chimaltenango, Sololá, Suchitepéquez y Escuintla. Su extensión aproxi-

¹ No haremos referencia a la clínica y al diagnóstico, porque han sido tratados ampliamente por numerosos autores, tanto nacionales como extranjeros.

mada es de 900 km², con una población de 41.085 habitantes y un índice de infestación de 21,41 por ciento de la población (10.024 enfermos). En ésta área se encuentran los municipios de San Pedro Yepocapa y Patulul, en onde recientemente se han efectuado instalaciones de centros de salud, con secciones dependientes del Instituto de Enfermedades Tropicales, dedicadas exclusivamente al estudio y tratamiento de la enfermedad de Robles.

b) *Zona N.º 2* — Comprende regiones de los departamentos de Escuintla y Santa Rosa, con una extensión aproximada de 700 km², una población de 16.824 habitantes y un índice de infestación de 13,22 por ciento (2.224 enfermos).

Es de advertir que en opinión de Díaz, de León y Dalmat, estas dos zonas serían continuas, formando una gran zona oncocercósica, ya que indican haber encontrado pacientes en los lugares intermedios. La Sección de Oncocercosis estima que se trata de enfermos ambulatorios que se han infestado en zonas vecinas. En la actualidad se planea una encuesta epidemiológica para establecer la distribución real de la enfermedad de Robles en el país.

c) *Zona N.º 3* — Descubierta en 1945-47; se encuentra limítrofe con México y está localizada en el Departamento de Huehuetenango, en los municipios de Cuilco y San Pedro Necta. Tiene una extensión aproximada de 170 km², con una población de 3.400 habitantes y un 28 por ciento de infestados (935 enfermos).

B. — EPIDEMIOLOGÍA

a) *Incidencia* — Los caracteres ecológicos de las zonas oncocercosas de Guatemala corresponden a regiones boscosas de cultivos con sombra (generalmente fincas de café), donde existen numerosos riachuelos y corrientes pequeñas, con abundante vegetación en las riberas; se encuentran en la vertiente de la cordillera de los Andes que va hacia el Océano Pacífico y su altura oscila entre 1.200 y 5.000 pies.

Conforme los datos proporcionados por la Sección de Oncocercosis de Sanidad Pública, la zona endémica tiene actualmente una extensión alrededor de 1.800 km², con una población de 61.241 habi-

tantes y un número de enfermos de 13.183 (21,52 por ciento de la población).

b) *Factores epidemiológicos* — En Guatemala, la enfermedad de Robles se presenta tanto en niños como en adultos e indistintamente en hombres y mujeres. Predomina en los trabajadores indígenas de las zonas afectadas, pero no es raro encontrarla en mestizos y blancos. Respecto a la edad, una de las observaciones de Robles (4) señala haberla encontrado en una niña de 3 meses, no siendo infrecuente verla en casos menores de 1 año. Ataca por igual a jóvenes y adultos. Los casos de ancianos infestados son también numerosos.

c) *Vectores* — Los simúlidos transmisores de la enfermedad de Robles en Guatemala son: *Simulium* (S.) *ochraceum*, S. (S.) *metallicum* y S. (L.) *callidum*. «Otras especies que podrían ser consideradas como vectores posibles presentan poca importancia, porque son menos numerosas y tienen tendencia a infestar regiones más bajas de las zonas de oncocercosis, en donde la frecuencia de la enfermedad es claramente mucho más débil» — de León (11).

En la obra de Dalmat (8), publicada en 1955, refirió 41 especies de simúlidos en Guatemala, así:

S. (D.) *acatenangoensis* Dalmat, *Cnephia aguirrei* Dalmat, *Gigantodax aquamarensis* (de León), S. (D.) *ardeni* Dalmat, S. (E.) *aureum* (Fries), S. (B.) *benjamini* Dalmat, S. (H.) *burchi* Dalmat, S. (L.) *callidum* (Dyar y Shannon), S. (H.) *capricornis* de León, S. (H.) *carolinae* de León, S. (L.) *colvini* Dalmat, S. (H.) *delatorrei* Dalmat, S. (L.) *downsi* Vargas, Martínez y Díaz, S. (L.) *dugesi* Vargas, Martínez y Díaz, S. (H.) *ethelae* Dalmat, S. (S.) *jacumbae* Dyar y Shannon, S. (S.) *jobbinsi* Vargas, Martínez y Díaz, S. (S.) *komp* Dalmat, S. (H.) *larvispinosum* de León, S. (D.) *mathesoni* Vargas, S. (S.) *metallicum* Bellardi, S. (D.) *mexicanum* Bellardi, S. (H.) *microbranchium* Dalmat, S. (H.) *nigricornis* Dalmat, S. (S.) *ochraceum* Walker, *Cnephia pacheco-lunai* (De León), S. (S.) *parrai* Vargas, Martínez y Díaz, S. (D.) *pulverulentum* Knab, *Cnephia roblesi* (De León), S. (D.) *rubicundulum* Knab, S. (L.) *samboni* Jennings, S. (D.) *smartii* Vargas, S. (S.) *tricornis* de León, S. (L.) *trivittatum* Malloch, S. (L.) *veracruzianum* Vargas, Martínez y Díaz, *Gigantodax wrighti* Vargas, Martínez y Díaz, S. (D.) *yepocapense* Dalmat.

C. — TRATAMIENTO

Robles instituyó las bases del tratamiento quirúrgico, habiendo efectuado personalmente más de 1.000 extirpaciones de nódulos oncocercósicos (4); también inició ensayos quimioterapéuticos con inyecciones intravenosas de 914 a dosis fuertes y practicó inyecciones intranodulares con biyoduro de mercurio.

Del trabajo de Bernhard (15), quién durante dieciocho años ha sido Jefe de la Sección de Oncocercosis en Sanidad Pública, tomamos los siguientes datos:

«En el lapso comprendido entre 1915 a 1928, el Estado no tomó ingerencia en el combate de la filariosis, y los trabajos se redujeron a los efectuados por médicos llamados por diferentes finqueros para el tratamiento de algunos enfermos.

En el año de 1928, el Estado nombró una brigada para combatir la enfermedad, pero su trabajo fué bastante reducido, ya que se concretó a la pequeña zona de Guanagazapa, Escuintla, trabajo que se continuó en esta forma hasta el año de 1933.»

Al reorganizarse la Dirección General de Sanidad, en 1931, se encargó a la Sección de Parasitología la atención al problema de la oncocercosis; posteriormente, en 1935, se creó una sección específica para emprender una campaña sistemática. Infortunadamente, no siempre se ha contado con los recursos económicos suficientes, lo que ha obligado a las consiguientes limitaciones.

En los primeros años de la Campaña Anti-Oncocercosa (1935-38), el tratamiento predominante fué de inyecciones intratumorales, empleando sucesivamente: neoriodina (Díaz), eter-formolado (Muñoz Ochoa), atebrina (Hoffmann), hexil-resorcinol, violeta de genciana, aceite esencial de quenopodio (Díaz), azul de metileno (López Selva) y fucshina fenicada (Estévez) (16).

De 1936 a 1938 se practicaron inyecciones y extirpaciones. De 1939 a la fecha (1958), únicamente se ha practicado la extirpación de los nódulos como tratamiento sistemático.

El procedimiento que se sigue actualmente en la Campaña Anti-Oncocercosa es el siguiente:

- 1) Examen completo (cabeza y cuerpo) de todos los habitantes de la zona oncocercosa, incluyendo los lactantes.

- 2) El examen se practica como mínimun una vez por año, procurando hacerlo cada seis meses en las zonas más infestadas.
- 3) Extirpación sistemática, bajo anestesia local, de todos los tumores localizados.

El trabajo global de la Campaña Anti-Oncocercosa desde 1935 a 1957 se resume así:

- a) Número de exámenes practicados por año, desde 1935 a 1957: 802.403.
- b) Número de enfermos portadores de tumores, de 1935 a 1957: 120.190.
- c) Número de tumores extirpados, de 1935 a 1957: 185.424.

Es de advertir que a pesar del trabajo efectuado y el número de años transcurridos, el tanto por ciento de portadores de tumores sigue siendo elevado.

Ya indicamos como fué Robles quién primero buscó un medicamento que actuara sobre la *Onchocerca*, ensayando inyecciones de 914 e inoculando nódulos con biyoduro de mercurio. Posteriormente, autores guatemaltecos emplearon inyecciones intranodulares en gran escala, utilizando diez medicamentos diferentes.

Al conocerse en el país los resultados obtenidos por Mazzotti y colaboradores, se procedió a efectuar tratamientos con hetrazán, los cuales hubo de suspenderse por las reacciones alérgicas que provocaron, lo que motivó que los pacientes rehuyeran dicho tratamiento. Poco tiempo después, Burch y colaboradores (18) efectuaron tratamientos con suramina sólo o alternándola con hetrazán. Finalmente, en el curso del año pasado, 1957, se hicieron ensayos con TWSb (dimercaptosuccinato de antimonio) (19).

Estamos a la expectativa de un medicamento que, teniendo acción específica sobre la *Onchocerca* adulta y las microfilarias, sea de un empleo práctico en el medio rural, ya que las condiciones socio-económicas de los pacientes hacen difícil trasladarlos a centros hospitalarios para someterlos a tratamientos quimio-farmacológicos que exigen exámenes de laboratorio y cuidados asistenciales.

D. — PREVENCIÓN

En el terreno preventivo, debemos confesar que no se ha tomado medidas contra los vectores, porque aún no contamos con un procedimiento práctico y económico que haga factible su empleo. Consideramos que es hacia este objetivo que deberá enfocarse los futuros planes de la campaña contra la oncocercosis y en nuestro criterio sería conveniente que la experiencia y recursos que actualmente se ocupan en la Campaña de Erradicación de la Malaria, al cumplir dentro de dos a tres años el proyecto en marcha, se emplearan en la erradicación de otras enfermedades transmitidas por insectos, que como la enfermedad de Robles y la enfermedad de Chagas son causa de graves problemas médico-sociales en el continente americano.

III. — CONCLUSIONES

1.^a La enfermedad de Robles lleva ese nombre en honor a su descubridor en el continente americano en 1915, quién presentó un estudio completo del problema: describió el parásito, atribuyó su transmisión por insectos del género *Simulium*, trazó magistralmente el cuadro clínico y señaló por primera vez las lesiones oculares.

2.^a Corresponde a Robles la prioridad de haber iniciado tratamientos quimio-farmacológicos y de haber sentado las bases del tratamiento quirúrgico; asimismo indicó que las microfilarias probablemente estarían en la dermis, hecho comprobado por Montpellier y Laigret en 1921.

3.^a La primera descripción de las lesiones oculares de la enfermedad de Robles fué hecha en 1918 por el Dr. Rafael Pacheco Luna, notable oftalmólogo guatemalteco.

4.^a La enfermedad de Robles tiene en Guatemala la misma distribución de hace 10 años: la zona endémica es de 1,800 km², con una población de 61,241 habitantes y 13,183 enfermos (21,52 por ciento de la población).

5.^a De 41 especies de simúlidos que han sido identificados en el país, solamente a 3 se les considera de importancia como transmisores de la enfermedad de Robles: *S. (L.) callidum*, *S. (S.) metallicum* y *S. (S.) ochraceum*.

6.^a En la Campaña Anti-Oncocercosa se practica examen completo de cabeza y cuerpo a todos los habitantes de la zona endémica, como mínimun una vez por año, y se extirpa bajo anestesia local todos los tumores encontrados.

7.^a A pesar de numerosos ensayos quimioterapéuticos, se está a la expectativa de un medicamento que a sua acción especifica contra la *Onchocerca* adulta y microfilarias una cualidades que le hagan de empleo práctico en el medio rural.

8.^a Se propone que al terminar el Plan de Erradicación de la Malaria, el personal y recursos económicos necesarios sean empleados en planes de control y si fuere posible de erradicación de otras enfermedades transmitidas por insectos: enfermedad de Robles y enfermedad de Chagas.

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STUDIES ON ONCHOCERCIASIS IN LIBERIA

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Liberia is a region where onchocerciasis is prevalent but where no cases of blindness due to this disease are known to exist. Previous studies have shown that infected individuals with relatively high skin microfilarial counts are not uncommon and that the characteristic skin lesions of onchocerciasis occur.

The present study was concerned with a quantitative analysis of the microfilarial distribution in the skin of onchocerciasis patients and its relationship to clinical manifestations of the disease. At the same time the efficiency for diagnostic purposes of the skin biopsy technic was compared with the skin scarification technic.

MATERIALS AND METHODS

A total of 184 patients (65 females and 119 males) were studied in the laboratory. Patients were stripped and carefully examined for the presence of onchocercomata and characteristic skin changes. The following five sites from the left side of the body were selected for

¹ The authors wish to thank Dr. Franc B. Livingstone for his help in the mathematical analysis.

investigation: outer canthus, shoulder (suprascapular), the flexor surface of the forearm, the hip (above anterior superior iliac spine) and the calf. At each of these sites both a scarification smear and a skin biopsy were taken. Skin snips removed at biopsy were weighed on an analytical balance; the weight generally ranged from 1-4 mg. Microfilariae were counted as they escaped from snips into normal saline until they ceased to emerge, the tissue was then transferred to a fresh drop of saline and teased apart for demonstration of microfilariae remaining. All microfilarial counts were expressed in number of larvae per milligram of tissue.

RESULTS

Table 1 summarizes the results obtained with the skin biopsy technique.

TABLE 1 — *Microfilaria counts at five body sites*

Site	Number examined	Number positive ¹	Total number mf.	Average number mf. / pos. biopsy	Average number mf. / subject
Eye	184	80 (43)	338	4.2	1.8
Shoulder . . .	184	146 (79)	2,695	18.5	13.5
Hip.	184	175 (95)	12,195	69.7	66.3
Calf	184	169 (92)	10,559	63.7	57.4
Forearm . . .	184	119 (65)	2,264	19.0	12.3
All sites. . . .	—	—	28,051	—	30

¹ Percentages in parenthesis.

It will be seen that 175 patients (95 percent) were found to harbor microfilariae in the hip, 169 (92 percent) in the calf and 80 (43 percent) in the outer canthus. But, whereas the average numbers of microfilariae per positive biopsy were 69.7 and 63.7 for hip and

calf respectively, there were only 4.2 at the outer canthus. The mean counts for both shoulder and forearm were intermediate and approximately equal.

Table 2 compares the efficiency in terms of recovery of microfilariae of scarification smears against biopsies.

It will be readily seen that skin scarifications are very much less reliable than skin biopsies for both diagnosis and recovery of microfilariae. When all five sites were scarified, only 63 percent of the subjects showed microfilariae—as compared to 100 percent with biopsies. The number of microfilariae seen in scarification smears furnishes no reliable guide to the number to be found on biopsy.

TABLE 2 — *Efficiency of scarification smears compared against biopsies*

Site	Total number	Scarification number positive ¹	Biopsy number positive ¹	Efficiency scarification/biopsy
Eye	184	3 (2)	80 (43)	.04
Shoulder	184	24 (13)	146 (79)	.16
Hip	184	92 (50)	175 (95)	.53
Calf	184	68 (37)	169 (92)	.40
Forearm	184	30 (16)	119 (65)	.25
Hip and calf	184	113 (61)	—	—
All combined	184	115 (63)	184 (100)	.63

¹ Percentages in parenthesis.

134 subjects (73 percent) showed no skin changes that were due to onchocerciasis. The 50 adults (27 percent) who did show skin changes were divided into two groups: 1. Early changes (i.e., changes confined in extent and degree)—31 subjects (17 percent); 2. Moderate to advanced changes—19 subjects (10 percent).

Table 3 compares the microfilarial counts, at the five sites, and the overall average counts, in these 3 groups of skin involvement.

The average microfilarial density in subjects showing no skin changes was close to that seen in subjects with moderate to severe

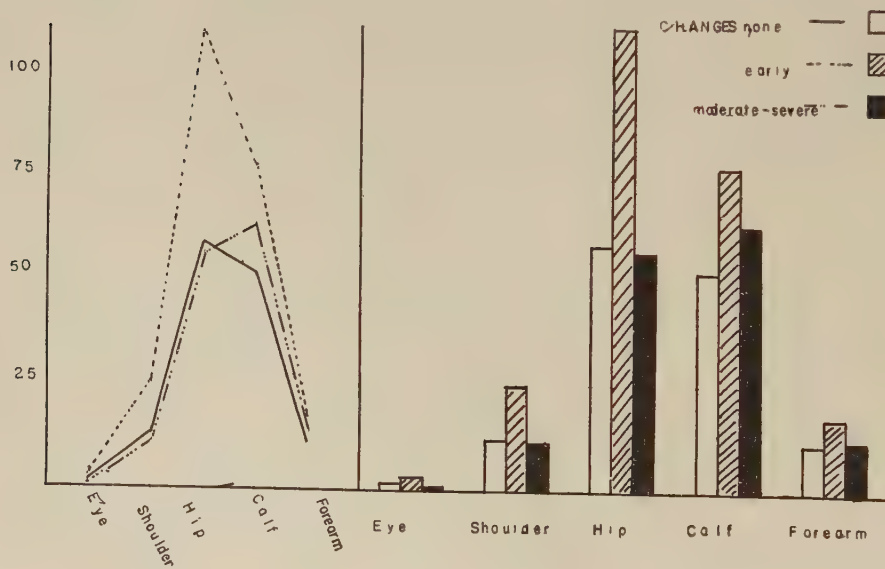
changes. (The average for the five sites combined was slightly higher in the latter group.) However, highest average counts were seen in

TABLE 3 — *Microfilarial counts in three groups of skin changes*

Skin changes	Number	Microfilarial counts — Biopsies											
		Canthus		Shoulder		Hip		Calf		Forearm		Total	
		Number	Average	Number	Average	Number	Average	Number	Average	Number	Average	Number	Average
None . . .	134	227	2	1,687	13	7,785	58	6,959	52	1,486	11	17,989	27
Early . . .	31	90	3	787	25	3,342	108	2,396	77	548	17	7,888	47
Moderate to advanced	19	21	1	223	12	1,068	56	1,204	63	230	12	2,774	29

cases having early skin changes. Skin changes were higher in females. Women comprised 30 percent of the whole series but 48 percent of

Mf. counts at five body sites in 3 groups of skin changes



those with skin changes. 55 percent of patients with light changes and 37 percent of those with moderate to severe changes were female. There were 148 adults (both sexes) under and 36 above 45 years of age. In those under 45 years 19 percent had early and 6 percent moderate to advanced skin changes; the comparable figures for those above 45 years were 17 percent and 28 percent, respectively.

Of the 184 patients examined, 80 (43 percent) showed microfilariae in the skin at the outer canthus. All patients were examined for evidence of eye involvement due to onchocerciasis — but no cases of blindness or obvious lesions were found. In 8 patients showing high microfilarial counts at the outer canthus, conjunctival biopsies were taken from the bulbar conjunctiva — and were all found positive for microfilariae. However, on examination with the corneal microscope none of these patients showed any microfilariae in the eyes.

DISCUSSION

The sorrelation coefficients may help to show a clearer pattern of the distribution of the microfilariae.

TABLE 4 — *Correlation coefficients of microfilarial counts for various sites*

Site	Outer canthus	Shoulder	Hip	Calf
Shoulder	.2445	—	—	—
Hip.	.1865	.5928	—	—
Calf	.2270	.5549	.7597	—
Forearm	.2891	.6468	.6236	.7178

While none of these coefficients is higher than .8000, they are all, with the exception of those pertaining to the outer canthus, above a value of .5540. In other words, the counts at the outer canthus are fairly independent and do not fit into the general «pattern». It is worth noting that the correlation coefficient for outer canthus and

forearm (.2891) is higher than that for the shoulder (.2445). The highest coefficient is that for hip and calf (.7597).

As to eye lesions due to onchocerciasis, not only have we not come across any lesions in our series, but we have not seen or heard of such cases in Liberia. It seems certain that the mere presence of microfilariae in the conjunctiva does not signify that specific eye lesions will occur.

SOME ASPECTS OF THE STUDY OF *SIMULIUM* *DAMNOSUM*

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Little more than ten years ago the control of *Simulium damnosum* Théobald seemed impossible and research on its habits offered little hope. Since then several campaigns against it have been so successful that it is appropriate to consider briefly some of the general objectives of further study. Afterwards some aspects of the biology and study of the species will be considered.

SOME OBJECTIVES IN THE STUDY OF *SIMULIUM DAMNOSUM*

Three subjects which seem to deserve special consideration are the improvement of existing control measures, the amount of control necessary in certain rural areas, and the possibility of resistance to insecticides.

With regard to existing control methods, further knowledge of the use of insecticides in running water and of the biology of the larvae would be useful. Onchocerciasis is mainly a rural disease in Africa because few towns are situated near large rapids and most of those so placed can be fully protected. In many rural areas extermination or other permanent control measures are impracticable owing to the expense of skilled staff, transport and road-building, and only partial or intermittent control are feasible. There is reason to hope,

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however, that these will be sufficient to prevent onchocercal blindness (Gordon, 1955), but it is important to know how much the fly population should be reduced. Resistance to insecticides would increase the need for further knowledge of the insect.

As far as biological studies are concerned, the answers to these problems seem to lie in the broad objective of a further general study of the species, with emphasis on certain aspects, particularly infective density. There is still much to be learnt about *S. damnosum* because it is not very long since the discovery of *Onchocerca* transmission stimulated work on this species, because the rural nature of the disease has hindered study, and because this has been largely centred in certain areas in accordance with the rather puzzling localization of onchocercal blindness.

It is usually difficult to find living accommodation and laboratory facilities for the study of the major vector of this rural disease, so it is usually necessary to adopt methods suited to field conditions.

DISTRIBUTION

Northern limit

The recent finding of onchocerciasis in people from the Yemen (Fawdry, 1957), where the vector is unknown, focuses attention on the known northern limit of *S. damnosum* across the Red Sea in the Sudan Republic, a neighbouring part of the Ethiopian zoogeographical region. In the northern Sudan *S. damnosum* is confined to the Nile and its large tributaries. In the Marra Mountains, where the conditions appear to resemble those in the Yemen to some extent, the streams contain countless millions of larvae of *S. elgonensis* form *hargreavesi* Gibbins, which does not bite man there.

Absence or scarcity in some apparently suitable places

There are some rapids, in regions inhabited by *S. damnosum* where this species appears to be absent or very scarce. Examples are the Fola rapids, on the Nile, the river Aswa and a stream near

Kapoeta, all in the Sudan (Lewis, 1953), and the great falls on the river Gurara (mentioned by Crosskey, 1956, p. 38; 1937) and some neighbouring streams in Nigeria. In such places it is important to be certain that the species really is absent or scarce and not merely difficult to find, as it is in some infested localities. The explanation of the failure of *S. damnosum* to utilize certain large rapids might reveal some features of its biology which could be used in control. Very fast water can tear larvae from their hold, but there are always eddies and backwaters near the rapids where current is reduced. Most of the areas considered have few or no human inhabitants or domestic animals and it is quite likely that the adult flies are unable to obtain mammalian blood for the development of eggs. In some ways *S. damnosum* is comparable with tsetse flies, some of which are notably affected by the distribution of their hosts. Owing to the difficulty of finding resting *S. damnosum* for the examination of stomach contents, little is known about the importance of their non-human hosts, of which there are several (de Meillon, 1957) in onchocerciasis areas, but there is little to suggest that they play an important role. Thick vegetation almost certainly keeps some females away from certain oviposition sites. Apart from these conjectures on the subject, we may note that certain individuals found in uninhabited places do show definite signs of having lived for some time without a blood meal (Lewis, 1958c), and that the falls at Wad Arud, on the river Atbara, where few adult *S. damnosum* were found in November 1958, are in an uninhabited part of the Sudan.

At Kapoeta, where there are plenty of people, the waterfall is a small isolated one and any larvae hatching at its head might have drifted down into calm water and died.

THE PREVALENCE OF LARVAE AND PUPAE

In many parts of Africa one can easily find the early stages of *S. damnosum* in any large rapid but not in other types of water. There are, however, exceptions to this rule; *S. damnosum* may be scarce or absent in likely-looking places, larvae and pupae may be numerous but difficult to find, or they may be numerous in places other than large rapids. The first of these anomalies is discussed above

and the other two below. In studying breeding places it is important to bear in mind that the larvae are to some extent a moving population and, unlike those of mosquitoes, may be found far from the point where the eggs were laid; it is well known that many *Simuliid* larvae float downstream, and the finding of *S. damnosum* pupae in part of the Tonkolili (Lewis, 1958c) and other places which adults could not have reached suggests that this species is no exception to the rule.

THE DIFFICULTY OF FINDING EARLY STAGES IN SOME AREAS

In several areas, such as Mvolo, in the Sudan, the Tonkolili valley, in Sierra Leone, and Lokoja, in Nigeria, where adults of *S. damnosum* were numerous and onchocerciasis prevalent, it was generally difficult to find more than a few larvae or pupae unless a fall in water level, particularly at the beginning of the dry season in the first two areas, happened to expose a particular patch of vegetation. Probably they tend to concentrate in inaccessible places. This difficulty in finding the early stages shows how much remains to be known about them and hinders their study. Much could probably be learnt by using the experimental cones developed in Canada. When there is not enough time for the systematic use of these cones, tow-nets might give valuable information about the moving population of larvae. I have used nets in two places, in the Blue Nile at Khartoum in search of *Chironomid* larvae, and below a breeding place of *S. damnosum* at Lokoja, in Nigeria. At Khartoum *Simulium* larvae were found at a point where they were not known to exist, and at Lokoja none were found in a short period.

UNUSUAL BREEDING PLACES

In contrast to the difficulty in collecting larvae and pupae is the finding of many of them in places where one might not expect to see any — places very different from the large rapids in which the species typically breeds. Examples in the Sudan are the placid waters of the Blue and main Niles and some very small falls in the south

(Lewis, 1948, 1953). Presumably the normal oviposition sites were not available, either because the strong flight of the adults had carried some far from their breeding grounds, or because the onset of the dry season had obliterated large rapids.

THE NULLIPAROUS AND PAROUS COMPONENTS OF A POPULATION OF FLIES

Nulliparous and parous caught flies differ from each other in many ways, in external and internal appearance, in physiology and in behaviour (Lewis, 1958*b*); and parous flies, having had a blood meal, are likely to include virtually all those containing infective *Onchocerca*. It is useful therefore to think of a population of female flies as composed of two distinct components, the nulliparous and the parous individuals, and, when examining a fly, to see if it has laid eggs, as well as determining its species and sex before proceeding to study other features.

VISIBLE DIFFERENCES BETWEEN INDIVIDUAL FLIES

The difficulty of finding resting flies, the unnatural behaviour of captive individuals, and other problems hinder the study of *S. damnosum*, but fortunately investigations are assisted by many visible changes which occur in the internal organs of an individual fly (Hinton, 1953; Lewis, 1958*b*) — too many to record in practice — and furnish much information about individuals and populations. No two dissected flies look exactly alike, and dissection affords a mine of information about the life of the species and one which can easily and quickly be used in the field with little apparatus. In particular, it tells us much about the age of flies and therefore about their capacity for disease transmission.

The process of recognizing parous flies (Lewis, 1958*b*) is likely to be quickened by the use of detergent or blue light which were employed by Lewis (1957, 1958*a*) and Colless (1958) respectively for mosquitoes.

TIME OF BITING

Nulliparous and parous flies have different biting cycles (Crosskey, 1958; Lewis, 1956, 1958*bc*), as some mosquitoes appear to do (Mattingly, 1957), with the result that risk of infection depends on time of day, and surveys of infection rates must be planned accordingly.

SURVIVAL DURING THE UNFAVOURABLE SEASON

It is difficult to see how *S. damnosum* survives the unfavourable time of year, usually the dry season, in places where it vanishes and quickly reappears in the adult stage. Crisp (1956) has discussed various theories, with special reference to the possibility that the adult may aestivate, and reappear when the air becomes moist, and he suggested (p. 57) that increasing humidity, rather than rise of river level, might account for certain increases in the number of adults. Muirhead-Thomson's (1957) work indicates that the egg probably does not aestivate, and de Meillon (1957) suggests that stranded pupae may survive for some time.

Some observations were made at Mvolo at the end of the dry season of 1948 (Lewis, 1953), and I have re-examined the notes made at that time, in the light of the above references (Table). The number of adults dissected and the percentages containing developing *Onchocerca* were:

May 13-19	49	0	May 21	48	10.4
May 20	23	13.0	May 22-29	140	2.1

The period covered by these observations can be divided into three. Shortly before the river began to flow a few adults could be found, all among trees near the bank. Then four events happened almost simultaneously: heavy rain fell, humidity noticeably increased, the river began to flow, and adults became common but not very numerous. This second phase persisted for about 13 days, during

which adults far from the river were not abundant and, like some in the Tonkolili valley at the end of the rains (Lewis, 1958c), probably represented the non-riverain proportion of a small population. Finally, adults attained their normal rainy season abundance, possibly because a new generation had bred in the river. The sequence of events was consistent with the theory of aestivation by adults, but the difficulty of finding them in very dry weather is still puzzling.

Some observations at Mvolo, in the Sudan Republic, when the river rose after the dry season of 1948

May	Rain millimeters	River level ¹	Notes
12	Trace	—	Sandy river bed with a few pools; no <i>Simulium damnosum</i> seen.
13	3.5	—	3 <i>S. d.</i> , call among trees, caught by 5 people in 3 hours.
14	2.9	—	1 <i>S. d.</i> brought by a resident.
15	56.8	—	No <i>S. d.</i> seen (no special search); river flowed in evening.
16	0	10.36	River flowing, air very damp; 18 <i>S. d.</i> brought by a resident.
17	0	11.00	A resident complained of <i>S. d.</i> , 42 caught near river in 30 minutes by untrained man.
18	0	11.65	<i>S. d.</i> not uncommon.
19	17.5	12.26	A few <i>S. d.</i> seen.
20	Trace	11.54	Some <i>S. d.</i> found near river.
21	Trace	11.27	A few <i>Simulium</i> larvae found in river.
22	0	11.84	Some adult <i>S. d.</i> seen.
23	15.5	12.06	Pupa skin of <i>S. d.</i> on stranded stick.
24	0	11.58	<i>S. d.</i> still few away from river.
27	99.3	10.98	Pupa of <i>S. d.</i> found.
29	34.5	12.58	Adult <i>S. d.</i> numerous about now.

¹ Metres above zero of gauge.

We know, however, that adults feed largely on plant sugars, that some apparently live a considerable time without sucking blood (Lewis, 1958c), and that adults can be difficult to find in places where they are numerous; and it is conceivable that many survive in secluded crannies out of contact with man. The first flies to be

dissected proved to be free of infection, but so did many of those examined after the first group of infected flies.

The finding of a pupa skin 7 days after the river rose suggests that some pupae may have floated down on drift-wood and helped to swell the adult population.

The situation in the northern Sudan is unusual in that *Simulium* vanishes in the rains when the river water is thick with silt. The method of survival is unknown.

FINDING THE *ONCHOCERCA* INFECTION RATE

One can not examine a sample of resting flies, as of endophilic mosquitoes, and it is necessary to collect biting females and to decide when to do so. This is rather like deciding when to study the human population of a village in which one could only examine people in the street. According to the time of day, one might encounter mainly children, mainly adults, or few people at all. It is usually impracticable, and unnecessary, to sample the *Simulium* population throughout the day, and, when looking for infected flies, one can save much time by examining parous flies only. These may be caught at a time of day when they are particularly active and it is to be hoped that the consequent elimination of a few parous flies biting at other times will not materially affect the result. This procedure makes it possible to examine many flies and to compare the results with infection rate obtained elsewhere in the same way.

THE NUMBER OF INFECTIVE *ONCHOCERCA* IN A FLY

Many microfilariae are entrapped by the peritrophic membrane (Lewis, 1953) and it would be instructive to know what determines the number which escape this fate and may survive to influence the infective density. Some microfilariae presumably enter the proboscis during a blood meal and avoid being swept backward by the passage of blood. Some are seen, at times, clinging to the proboscis after a blood meal and may perhaps enter the gut after the peritrophic membrane has been formed.

S. DAMNOSUM WITHOUT ONCHOCERCIASIS

Human onchocerciasis is sometimes found for the first time in an area where a potential vector has long been known. Nine cases, discovered in the Northern Province of the Sudan (Satti and Kirk, 1957), make it necessary to modify previous ideas about this area. If the disease proves to be rare, the discovery, by showing that the disease has reached so far north, will stress its failure to become firmly established over a long northern riverain area. A previous theory about the lack of transmission in this region (Lewis, 1953), although quite possibly correct, needs to be reconsidered in view of the necessity of collecting flies, intended for dissection, at a particular time of day.

SUMMARY

Some general objectives of future work are briefly discussed.

Some aspects of the study of distribution, breeding habits, nulliparous and parous flies, visible changes in individual flies, time of biting, aestivation, and infection rates are considered.

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PRELIMINARY RESULTS OF A TRIAL OF ANTIMONY DIMERCAPTOSUCCINATE OR «TWSb» (FRIEDHEIM) AGAINST INFECTION WITH *ONCHOCERCA VOLVULUS*

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Dr. E. A. H. Friedheim (personal communication) has tested the effect of TWSb on cases of onchocerciasis in the Sudan Republic and has found it to be both microfilaricidal and capable of blocking the embryogenesis of adult female worm by a destruction of unhatched embryos. In 1957 Dr. Friedheim kindly sent a supply of the drug to the author for testing in the Cameroons, and the preliminary results of this trial are described in the present paper.

MATERIALS AND METHODS

Ten adult male African volunteers of the Bakundu tribe were selected to take part in the trial. All weighed over 45 kg and they all showed infection with *O. volvulus*. Prior to treatments they were subjected to a general physical examination to exclude gross systemic disease, their haemoglobins were ascertained to be over 12 g percent, and they were each given 300 mg of mepacrine to suppress any overt malaria, and 1 g of hoxylresorcinol to evacuate any *Ascaris* that were present.

The intensity and distribution of *O. volvulus* microfilariae in their skins were then assessed by the method of multiple weighed skin snips (Kershaw, Duke and Budden, 1954). Snips were taken from the ankles, calves, buttocks, scapulae and deltoids on both sides and

from the right outer canthus. The eyes were examined, and the numbers of microfilariae visible in the anterior chambers against the fully dilated pupils were counted using a plus 32 lens in the ophthalmoscope.

The patients were treated in their villages. 0.4 g of powdered TWSb was dissolved in 4 cm³ of the recommended diluent (pyrogen-free distilled water containing 0.7 percent acetic acid) supplied with the drug and the total volume in the syringe was then made up to 10 cm³ with 20 percent glucose solution. Injections were given intravenously every day for 6 or 7 days, the total dosage being 2.4-2.8 g.

Two weeks after finishing this course the patients were re-examined in the same manner as before treatment began, the skin snips being taken from within 1 cm of the previous scars. One year later a further similar examination was made and it is these results which are recorded in the present paper. Final assessment of the effects of the drug on this series of patients will be made after further re-examination 2 years subsequent to treatment.

During the follow-up period the volunteers were exposed to the normal amount of transmission of *O. volvulus* which pertains in the environs of their village, and to which they had been exposed throughout their lives.

RESULTS

1. *The concentration and distribution of microfilariae in the body before and after treatment*

Table 1 shows the microfilarial densities found in the 10 patients at the examinations made before, 2 weeks after, and 1 year after treatment. The figures given are (a) the number of microfilariae seen in both anterior chambers, (b) the total number of microfilariae per milligram of skin present in the 5 snips taken from above the waist (2 scapulae, 2 deltoids and the right outer canthus), (c) the total number of microfilariae per milligram of skin present in the 6 snips taken from below the waist (2 buttocks, 2 calves and 2 ankles), and (d) the total number of microfilariae found.

Table 2 shows, for the whole series of 10 patients, the geometric mean concentrations of microfilariae in the various parts of the body

before and after treatment, with the after-treatment figures expressed as percentages of the pre-treatment concentrations.

2. *Toxic and untowards effects occurring during the course of TWSb*

Table 3 shows the various toxic and untoward effects experienced by the 10 patients during and immediately after their course of treatment.

DISCUSSION

1. *The effect of TWSb on Onchocerca volvulus*

The results shown in Table 1 and 2 demonstrate that TWSb has a marked and rapid microfilaricidal effect comparable indeed with that of diethylcarbamazine. In 2 patients the immediate clearance of microfilariae was complete; in the 8 others it was partial, but in every case by the end of treatment the overall concentration of microfilariae in the body had been reduced to less than 50 percent of its pre-treatment level. It will also be noted from Table 2 that there is a tendency for the immediate reduction in microfilarial numbers to be greatest where their original concentration was highest and that there is a lesser reduction in the numbers of microfilariae situated toward the extremities of the body than among those distributed centripetally. In this last respect the microfilaricidal effect of TWSb is in direct contrast to suramin, which acts most rapidly on microfilariae situated centrifugally (Duke, *in preparation*). This suggests that perhaps TWSb acts primarily on younger microfilariae and that suramin has a greater effect on older microfilariae.

From the results of the re-examination made 1 year after treatment we may assess to some extent the effect of the drug on the adult worms, although a more accurate assessment must await the 2 year follow-up. The first 2 patients in Table 1 (Mbinda and Mallo) and for all practical purposes the third patient (Mbonda) showed an immediate complete elimination of microfilariae with no return 1 year later. This finding implies either that all the adult female worms have been killed or that their embryogenic activity has

been severely and probably permanently disorganised. It is also apparent from a study of 4 other cases (Ndifor, Mukoko, Busaka, and Diange), whose microfilariae were not entirely eliminated and who showed some build-up over the ensuing year, that the rate of build-up was in general much slower than would have been expected after a drug such as diethylcarbamazine, which is believed to have no effect on adult worms. By extrapolation the build-up to pre-treatment levels in these patients would have taken between 5 and 17 years, whereas after diethylcarbamazine the microfilarial population may be expected to re-establish itself at its original level over an interval of 1 to 3 years (Duke, 1957).

In the remaining 3 cases (Ndi Sona, Mudika and Ebong) the rate of build-up was such that by extrapolation the pre-treatment concentration of microfilariae would have been reached after 18 months to 3 years, a time lag comparable to that occurring after diethylcarbamazine.

It has also been shown (Duke, 1957) that after diethylcarbamazine the distribution of the regenerating microfilarial population is essentially similar to the pre-treatment pattern. It will be seen however from Table 1 that the post-treatment build-up in Mukoko, Ndi Sona and Mudika has been confined to the lower half of the body and that the concentration above the waist has remained static or declined. Similarly Diange's post-treatment microfilarial build-up is all above the waist with a decline in the lower parts of the body. We may conclude from this distribution of returning microfilariae that in these patients some of the adult female worms had been killed or sterilised while others were still fecund.

2. Toxic and untoward effect of TWSb

All patients complained of fever and pruritus beginning a few hours after the first injection and persisting in varying degrees throughout the course. The local reactions affected most severely those parts of the body where microfilariae were present in large numbers, namely the lower limbs. They were characterised by itching, dermatitis and swelling of the affected parts followed by desquamation, and they resembled in all respects the reactions seen after administration of diethylcarbamazine save only that they were milder

TABLE 1 — Showing the number of microfilariae of *O. volvulus* found per milligram of skin in 10 volunteers before and after treatment with TWSb

[illegible]

TABLE 2—Showing, for a series of 10 volunteers, the geometric mean concentrations of microfilariae of *O. volvulus* in various parts of the body before and after treatment with TWSb

Position of microfilariae	MG		MG		MG	
	before treatment		2 weeks after treatment		1 year after treatment	
Anterior chamber	0.53	100 %	0.45	84 %	0.11	21 %
Right outer canthus	3.0	100 %	0.6	20 %	0.6	20 %
Deltoids	24.5	100 %	2.1	8.6 %	4.1	18.5 %
Scapulae	39.9	100 %	3.1	7.8 %	4.8	12.0 %
Buttocks	162.6	100 %	6.8	4.2 %	14.7	9.0 %
Calves	170.7	100 %	6.4	3.7 %	22.0	12.9 %
Ankles	55.4	100 %	6.5	11.7 %	11.3	20.4 %
Whole body	538	100 %	15.1	2.9 %	42.9	8.0 %

TABLE 3 — Showing the various toxic effects and untoward symptom experienced by 10 volunteers treated with TWSb

[illegible]

in degree. Two patients with high concentrations of microfilariae near the eye showed a conjunctivitis, and 3 patients complained of pain in existing nodules. It was considered that all these reactions could be attributed to the death or disorganisation of adults and microfilariae of *O. volvulus* in the body.

Three patients suffered from complete anorexia, severe vomiting, prostration and a feeling which they described as one of «impending death». They were treated with liberal doses of glucose intra-venously or by mouth, and with Largactil, and within a week of stopping the drug they were eating normally and on the way to recovery. This type of reaction occurred only in those patients who were almost or completely cleared of parasites as a result of treatment. The most reasonable explanation of this seems to be that these patients were unable to detoxicate and excrete the drug as well as the others and that consequently they suffered both from the toxic action of high concentrations of the drug and also from the concomitant increased destruction of parasites.

SUMMARY AND CONCLUSIONS

Preliminary results comprising a 1 year follow-up of 10 cases of onchocerciasis treated with 2.4-2.8 g of TWSb indicate that this drug has a marked microfilaricidal action and is also capable of sterilising or killing a number of adult female worms. The susceptibility of individual patients to the drug varies, but it appears probable that if a sufficiently high dosage is given, all microfilariae can be eliminated and there will be no regeneration over the ensuing year. The level of drug concentration which brings about complete eradication of the parasites over this period is very near the limit of human tolerance and leads to severe reactions which are doubtless exacerbated by the coincident rapid destruction of heavy worm-loads.

The results of this trial of TWSb are sufficiently promising to warrant the search for and trial of other closely related chemical compounds, which may be less toxic to man but equally effective against *O. volvulus*.

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OBSERVATIONS RELATING TO THE INSECTICIDAL CONTROL OF *SIMULIUM DAMNOSUM* THÉOBALD

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Twenty five years ago Gibbins and Loewenthal (1933) wrote that a study of the breeding habits of *Simulium damnosum* had «proved the futility of attempting to attack the fly in its early stages». It is hardly likely that anyone would now agree with this view, having in mind the later development of synthetic insecticides and the recent success of DDT as a larvicide on the Victoria Nile.

In recent years large scale operations against *S. damnosum* appear to have been effective in eradicating this insect from the Congo rapids at Léopoldville (Wanson *et al.*, 1949) and the Victoria Nile in Uganda (Barnley, 1953). These successes have stimulated further insecticidal control in other parts of Africa, and engendered the hope that eradication of *Simulium damnosum* might prove relatively easy with enormous resultant benefit in the elimination of human onchocerciasis.

Indeed, one wonders whether the remarkable success of these two control schemes has not induced an over-optimistic outlook with regard to the feasibility of eradicating, or at any rate easily controlling, *S. damnosum*, so that the problem has perhaps appeared to resolve itself into a comparatively simple matter of dosing a sufficient number of rivers in sufficient places over a suitable period.

But it is easily overlooked that the excellent results from the control schemes on the Congo and Victoria Nile were achieved in areas where the *S. damnosum* foci, albeit very large ones, were isolated; the results obtained in these areas may not therefore be any guide to control in the vast non-isolated areas where onchocer-

ciasis is endemic over several thousand square miles (e.g., Northern Ghana or southern Adamawa Province of Nigeria) and where the vector breeds almost continuously in whole river-systems. It is this type of area which presents the greatest challenge to entomological control, for while eradication of *S. damnosum* may be achieved in a few isolated foci, it may prove extremely difficult if not impossible in these widespread endemic areas where a multiplicity of breeding rivers exists, often in very inaccessible country.

Hughes (1956), in Ghana, has recognised this difficulty and states that, in his view, insecticidal control on a continent-wide scale will never be possible, although strictly local control may be. However, one of Hughes' reasons for this belief is that «*S. damnosum* can colonize tiny temporary streams» — which, although it may be true of Ghana, is certainly not true of Northern Nigeria. Nevertheless, one feels compelled to agree with Hughes that continent-wide control may never be possible, but any prognostication of this sort could prove mistaken — for no doubt it seemed to Gibbins and Loewenthal in 1933 that it never would be possible to attack *S. damnosum* in the larval stage.

Recent control work has not in general supported the early hopes of easy control or eradication. In the Mayo Kebbi eradication scheme in French Chad (Taufflieb, 1955, 1956) unexpected difficulties were encountered and *S. damnosum* survived the first attack upon it by combined simultaneous helicopter and ground applications of gamma-BHC, although the Mayo Kebbi focus is only 55 km long and is isolated by «une bonne centaine de kilomètres» from any other focus. In Northern Nigeria control has been attempted over the last three years on the periphery of a very large onchocerciasis area in Niger and southern Zaria Provinces in order to assess the practicability of local control in such a non-isolated area where eradication is out of the question, at least at present. This control work has shown that the adult *S. damnosum* population can be held down to a very low level by annual applications of DDT as a larvicide, but that even very low adult densities are adequate for rapid reinfestation of cleared rivers. Hence neither of these recent control schemes has fully borne out the early promise shown elsewhere.

There seems no doubt that control has mostly been applied with only sketchy and inadequate knowledge of biology of *S. damno-*

sum, and it is curious that a good deal of control has been put into operation (the more remarkable that so much success has been achieved) when we still really know very little of the detailed behaviour of *S. damnosum* and how this varies in different parts of Africa. We know, for example, next to nothing of the male sex, and little or nothing of the mechanism by which the fly survives the drought period in the drier savanna areas, even the theory of the drought-resistant egg being now generally abandoned in favour of an aestivating adult. Likewise, while there is no evidence in Northern Nigeria that *S. damnosum* can colonize tiny temporary streams, this is said to occur in Ghana; this point, which would clearly be of importance in practical control, suggests that the behaviour may well be different in different geographical areas. Experience in Nigeria makes it hard to accept that *S. damnosum* can «colonize tiny temporary streams» and the late E. G. Gibbins has long since pointed out that if it could exist in such situations Uganda would be overrun with the fly. But it is now becoming clear that *S. damnosum* is a markedly different insect in the savanna, where the rivers often dry, than in the forested areas, where the rivers flow perennially; it appears, for instance, that in the forest the adult fly does not disperse nearly as far from its breeding places. Such environmental differences in behaviour might well entail different methods of insecticidal attack.

While our inadequate knowledge of *S. damnosum* biology may suffice to enable us to deal with the occasional isolated focus, we may require to know very much more before we can embark with any prospect of success on insecticidal control in the more widespread infested regions where many practical difficulties also exist. These extensive onchocerciasis areas often lie in remote, inaccessible, regions (e.g., Adamawa Massif) where larviciding from the ground would be rendered extremely difficult and where the mountainous terrains would make aerial spraying hazardous. The latter mode of attack would also without doubt prove almost prohibitively expensive and perhaps economically unsupportable in relation to the small population which would benefit, since the most extensive onchocerciasis areas are normally ones of low population density from which little revenue derives.

To conclude, it seems to me timely to emphasise that, while the prospects appear to be good for control or eradication in the narrowly circumscribed *S. damnosum* foci, we ought not at present to be over-hopeful about the prospects for insecticidal control in the larger, more diffuse, onchocerciasis areas to which I have drawn attention. It may well prove in the long run that chemotherapeutic means will be more effective in the large scale destruction of onchocerciasis.

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GENERAL DISCUSSION

DR. PIERRE RICHEL (France) — Général Richet donne une idée de l'importance en Afrique française occidentale et équatoriale de la volvulose qui est bien, dans les principaux foyers où elle sévit, un véritable fléau souvent cachectisant, carenciel et aveuglant dont les répercussions sociales et économiques peuvent devenir catastrophiques.

Il s'étend sur le succès total ou partiel des campagnes d'éradication du vecteur simuliédu déjà menées au Tchad français et en des territoires étrangers — Congo belge, Kenya, Canada.

Il décrit la campagne menée dans les Mayo-Kebbi (Tchad) depuis 1954-1955.

Il expose enfin le problème particulier de la volvulose en Afrique Occidentale française, où de vastes enquêtes entomologiques, écologiques, hydrobiologiques sont patiemment, minutieusement menées depuis début 1956 dans les principaux bassins affectés en vue d'entreprendre vers 1960 une immense tentative d'éradication du vecteur par l'application de larvicides (D.D.T.).

DR. E. A. H. FRIEDHEIM (U.S.A.) — Dr. Duke's paper is a confirmation of my own earlier observations from the Sudan, indicating that TWSb affects the adult female onchocerca within the nodes. My own work provides more detailed information on the nature of this effect: antimony, in the form of TWSb, produces a necrosis of the intra-uterine embryos, in as far as they are contained in their shell. This necrotizing effect is particularly pronounced in the early microfilaria, coiled up in the shape of a bretzel. Thus, TWSb breaks the production line of microfilaria. This is a late effect, appearing only about 2 weeks after a TWSb course. The effect of the drug on the sheathless microfilaria in the nodes and in the skin is less clear and uniform. In some of my patients, a week treatment with TWSb was followed within 2 weeks by the disappearance of skin-microfilaria, as

in the 2 cases reported by Dr. Duke. In other patients I found up to 4 courses of TWSb, totaling over 10 g of TWSb, to have no significant effect on the count of microfilaria in the skin. This holds for an observation period not exceeding 3 months. It is quite possible, and I believe probable, that the skin microfilaria will disappear later, when they reach the term of their natural life span, as a result of the drug effect on the microfilaria production. I believe that the treatment of onchocerciasis with antimony, in the form of TWSb, opens the view on a causal therapy of this disease.

DR. M. H. SATTI (Sudan) — In the Sudan, we are interested in onchocerciasis because it affects a large area of Bahr el Ghazal and part of Equatoria provinces. These form our most southerly border.

It is interesting to hear this afternoon that doubts are being cast on the aetiology of the blindness and its relationship to onchocerciasis. We had entertained such doubts in 1948. Several factors were considered, amongst which were malnutrition and fish-poisons. Fish-poisons were thought to contain a rutin-like substance. All our results are so far negative. As to malnutrition, the status is the same in neighbouring localities where no blindness and no onchocerciasis are found.

It is observed that the distribution of the blindness coincides with that of the disease (onchocerciasis) as well as with that of the vector (in the Sudan it is *Simulium damnosum*).

As you have just heard from Dr. Lewis, this fly is prevalent in the Abu Hamad reach of the Nile, but no onchocerciasis had been discovered. In this connection, it is to be pointed out that Prof. Morgan last year (1957) found cases in this region. The eye manifestations in this part have yet to be sorted out, as trachoma also exists there.

Mac Kelvie in 1948 performed many eye operations in Bahr el Ghazal and microfilariae were found in the fluid from the anterior chamber.

In the endemic area, the blindness decreases and so also the disease as you go away from rivers where the vector abounds.

It is, therefore, considered that the association between the onchocerciasis and the blindness is a real one and is in no way accidental.

2ND SESSION: **Epidemiology and control of filariasis**

Saturday, September 6th—11:00 to 12:30 a. m. Room D

The meeting was held under the chairmanship of Dr. William Kershaw, in the absence of the appointed Chairman, Dr. J. Travassos. The Rapporteur was Dr. William Kershaw and the Secretary was Dr. Mário M. A. Abreu.

The following papers were presented and discussed.

**EPIDEMIOLOGY AND CONTROL OF FILARIASIS
WITH SPECIAL REFERENCE TO FRENCH POLYNESIA**

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INTRODUCTION

This symposium is designed to review the latest knowledge concerning the epidemiology and control of the filariae which parasitize man, and the selected speakers will summarize the findings of as many workers as possible from the several global areas indicated.

Apart from *Onchocerca volvulus*, reviewed in the preceding session, some 6 species of filarioidea parasitize man. *Wuchereria bancrofti*, *Wuchereria malayi* and *Loa loa* definitely produce symptoms while *Acanthocheilonema perstans*, *A. streptocerca*, and *M. ozzardi* are of questionable pathogenicity. Recently *W. bancrofti* var. *vauceli* from Madagascar has been described by Galliard and Brygoo (1955). *Culex*, *Anopheles*, *Aedes* and *Mansonia* transmit *Wuchereria*: *Crysops*

transmits *Loa loa* and *Culicoides* transmits *Acanthocheilonema* and *Mansonella*.

The following topics will be reviewed:

1. *The prevalence and severity of clinical manifestations*, especially elephantiasis, hydrocele and lymphangitis.

2. *The species of parasites* found in each area, their microfilarial prevalence and density rates per cubic millimeter of blood, or multiples, thereof. (1. and 2. displayed by age groups.)

3. Drugs employed in treatment or prevention.

4. The vectors and reservoirs of each parasite and their bionomics as applied to control. It was originally assumed that man was the only host of the human filariae, but Gordon (1955) and Kershaw (1955) indicate that monkeys are reservoirs of *Loa loa* and Edeson *et al.* (1955, 1957, 1958) in Malaya implicate several reservoir hosts of *W. malayi* and report its successful transmission to cats, *Macacca*, and the slow loris.

5. *Prevalence rates of the helminthic larvae* in vectors.

6. *The control or eradication of filariasis* is a subject that has lagged far behind its rightful place in our modern programs of tropical public health. Although this disease is highly disabling and produces conditions among the most bizarre and grotesque found in human medicine; although Stoll (1947) estimates it affects 189 million people throughout the world, and although it was the first human disease shown to involve an arthropod vector (see Manson, 1878), few control programs have been instituted to date.

Two world conferences on filariasis — the first sponsored by the South Pacific Commission in 1951 at the Institut de Recherches Médicales de l'Océanie Française in Tahiti and the second in 1955, a Study Section sponsored by the World Health Organization at the Institute of Medical Research in Kuala Lumpur, Malaya — have helped to summarize our knowledge on this subject. It is hoped that the current International Congresses on Tropical Medicine and Malaria will further stimulate activity.

During the last decade newer techniques of mosquito control and mass administration of diethylcarbamazine (hetrazan, banocide, notezine) have become available for control of filariasis. (See Hewitt, 1950 and 1950a.) Experiences involving the application either of one or of both methods will be reviewed. The discussion will be opened

by summarizing the results with non-periodic filariasis from French Polynesia.

FILARIASIS IN FRENCH POLYNESIA

A cooperative research and control project between the Institut de Recherches Médicales de la Polynésie Française in Tahiti and the Pacific Tropic Diseases Project of the School of Medicine, University of California, Los Angeles, has been in progress for some years. Prof. Henri Galliard directed the first survey (Galliard *et al.*, 1949 and 1949a). Subsequent investigations relating to epidemiology and the most efficient and rapid means of eradicating filariasis followed. (See Beye *et al.*, 1952, 1953; Kessel *et al.*, 1953; and Rosen, 1955.) A defined control program for the Society Islands was instituted in 1953. (See Kessel *et al.*, 1954, and Kessel, 1953a, 1957 and 1957a.) A brief summary follows:

I. EPIDEMIOLOGY. — Epidemiologic surveys involved man, the parasite and the vector.

1. *Man*. — Clinical surveys showed that elephantiasis occurred in 14 percent, hydrocele in 16 percent of males and lymphangitis in 27 percent of adults.

2. *The parasite*, «*Wuchereria bancrofti*».

(a) *Microfilariae in man*. — Blood surveys determine prevalence rates of microfilariae. Thick blood films made from 20 mm³ of finger blood are considered adequate for most surveys and control programs. In this study preliminary surveys in the Society Islands showed a microfilaremia of 32 percent.

It is also valuable to determine (a) frequency distribution of positives at selected microfilarial levels, e.g., 0; 1-5; 6-10; 11-30; 31-50; 51-100; and 100+ and (b) the density, or average number of microfilariae in a unit of blood per person, e.g. 20 mm³, either for all examined or per positive person. The average density of microfilariae per 20 mm³ for the whole population was 30, with a range from 17 to 48.

Kessel (1957) shows a closer correlation between density of microfilariae and elephantiasis from several areas in the South Pacific than between microfilaremia rates and elephantiasis. Rachou's surveys

(1954, 1955) on periodic filariasis in Brazil confirm this thesis, while those of McFadzean (1954) and de Rook (1957, 1957a) show variations.

(b) *Larvae in mosquitoes*.—Prevalence rates and the density rates of larvae of *W. bancrofti*, both for all stages of larvae and for infective stages, are determined by dissection of mosquitoes. Details of the procedures employed for mosquito surveys, including collections and dissections, were reported by Bonnet *et al.* (1956) and Kessel (1957a). The Table shows the results for *A. polynesiensis* in relation to blood surveys.

3. Vectors.

(a) *Mosquitoes of area*.—Rosen (1955), Kessel (1957a) and Belkin (personal communication, 1958) list the following mosquitoes in the Society Islands, i.e., (1) *Aedes aegypti*; (2) *A. edgari*; (3) *A. polynesiensis*; (4) *Culex annulirostris*; (5) *C. quinquefasciatus*; (6) *C. n. sp.*, reported as *C. litoralis* but undoubtedly a derivative of *C. jepsoni*; (7) *C. atriceps*, a derivative of the preceding which has invaded tree holes and small containers of water; and (8) *C. n. sp.*, a derivative of *C. atriceps* or of (6) *C. n. sp.* just mentioned. While *A. polynesiensis*, *A. edgari*, *C. quinquefasciatus*, *C. atriceps*, and *C. n. sp.* (8 above) may be infected in the laboratory with non-periodic type of *W. bancrofti*; in the field only *A. polynesiensis* (25 percent) and *C. quinquefasciatus* (1.2 percent), when caught near or in houses known to harbor carriers, were positive for all stage larvae. *A. polynesiensis* therefore is considered to be the most important vector of the area.

(b) *Aedes polynesiensis; its bionomics and prevalence*.—This mosquito is sylvatic in habit but also is peridomestic, occurring around houses located near shrubbery or trees. It breeds in small containers both natural and man-made, such as coconuts, tree holes, rock holes, land crab burrows, pirogues, tin cans, bottles or gasoline drums. The females feed in the day time on a variety of hosts such as man, horse, cow, dog, cat, pig, and rat. The dispersal range is considered to be short, usually not more than 100 m. It enters houses to bite, but does not often remain therein, resting rather in shaded vegetation or in crab holes, rat eaten coconuts, etc.

Determining mosquito prevalence rates by larval surveys or by catching adults in mosquito traps is not successful, but collecting on

Field results in 16 areas before and 3 years after recommended control measures were applied

Blood surveys			Mosquito surveys				
		Percent positive	Density per 20 mm ³	All stages of larvae		Infective stage larvae	
				Percent positive	Density	Percent positive	Density
Averages	{ Before . .	32.0	30.0	11.4 *	0.6	5.0	0.13
	{ After . .	3.2	0.6	1.7	0.07	0.3	0.016
Minimum and maximum. .	{ Before . .	24 to 44	17 to 48	7.7 to 18.7	0.2 to 0.8	2.0 to 9.0	0.06 to 2.0
	{ After . .	0.7 to 7.0	0.07 to 2.3	0 0 to 4.6 **	0.0 to 0.17	0.0 to 1.0 ***	0.0 to 0.04

* Mosquito survey averages before, from 13 districts only.

** 3 districts were negative.

*** 6 districts were negative.

Potential transmission index { Before . . 100 (in basic district).
 { After . . 3.2.

human bait by use of a suction tube is satisfactory. The average number of mosquitoes per dwelling caught per minute on a man who stands for 10 minutes in a shaded spot within 10 m of each house is termed the mosquito prevalence rate for an island or district. In uncontrolled districts this ranged from 0.16 to 2.56 with an average of 0.6 in 13 diversified areas.

This procedure of collecting mosquitoes adopted in the Society Islands, where the individual houses are scattered around the island, usually not far from the bush, may not be applicable to other areas of Polynesia, e.g., Samoa or Fiji, where people usually reside in villages, as reported by Jachowski and Otto (1953) and Symes (personal communication, 1956). Likewise, where other genera of mosquitoes are involved, e. g., *Culex* or *Anopheles*, which rest in houses and bite at night, collection techniques will be quite different.

(c) *Potential transmission index*.—In an attempt to evaluate transmission in this study, a potential transmission index was determined by multiplying the prevalence rate of mosquitoes of an area by the density of «all stage larvae» of *W. bancrofti*. This was corrected by an arbitrary factor of $\times 250$. An area in which careful observations were made for a period of 3 years before control yielded an index of 100 and this became the standard of comparison.

II CONTROL.—For preliminary epidemiological surveys and studies regarding safety, effectiveness and dosage schedules of diethylcarbamazine, see Beye *et al.* (1953) and Kessel (1957, 1957a). Current schedules and results will be summarized here.

1. *The program* includes mass-administration of diethylcarbamazine and mosquito control as follows:

(a) Perform spot clinical, blood and mosquito surveys in strategic parts of an area to provide a cross-section of the prevalence of filariasis.

(b) Treat all people in the area with diethylcarbamazine, once a month, for 12 months, with 6 mg per kilogram of body weight in a single daily dose.

(c) Institute mosquito larval control program, to be continued indefinitely.

(d) Six months after last diethylcarbamazine treatment, i.e., at 18 months, perform first complete blood survey.

(e) Administer diethylcarbamazine once a month for 1 year to all remaining positives and immigrants.

(f) One year after completion of (e), repeat follow-up blood survey on all persons, and perform mosquito survey.

(g) Continue diethylcarbamazine once a month to the few remaining positives until they become negative by annual blood survey.

2. This program is directed toward:

(a) *The definitive host, man.* — Measures are mainly educational involving (1) the youth in schools, and (2) the whole population, by instruction and supervision of measures relating to mass administration of diethylcarbamazine and elimination of mosquito breeding places. Distribution of pamphlets, use of posters, charts, lantern slides, motion pictures and radio talks are routine.

Voluntary cooperation has been the objective of this program. Fines may be levied if an individual fails to cooperate in the mosquito control program, but these have seldom been enforced. The few who miss treatment are (1) new immigrants to an area, (2) refused for personal reasons, (3) excused for medical reasons and (4) children under 2 years of age.

A criticism of using diethylcarbamazine for filariasis control is the allergic response which sometimes follows destruction of microfilariae. After observing the administration of diethylcarbamazine in the dosage of 6 mg per kilogram of body weight once a month to thousands of individuals, March and Laigret (1958) conclude, in general, this dosage is not regularly accompanied by undesirable side effects. Subsequent doses either fail to elicit a reaction or the reaction is less severe.

Reactions following the first dose of ortequine may be of two types. (1) A transitory fever, dizziness or nausea which occurs in some 20 percent of the people treated and lasts from a few hours to 2 or 3 days. Antihistamine or aspirin may be administered. (2) In about 1 percent, a delayed reaction with a bullous eruption involving one or more limbs occurs. Cortisone or prednisone in a total dosage ranging between 50 mg and 100 mg induces complete recovery in from 1 to 3 days.

As there are no attempts in this program to evade or minimize the fact that reactions to diethylcarbamazine may occur, full confidence of the people is maintained throughout the program.

Treatment of people showing clinical signs of filariasis is provided routinely in clinics, during the control program.

(b) *The parasite, «W. bancrofti».* — By the procedures described above, marked reductions in microfilaraemia and density of microfilariae occur within 3 months and most individuals are negative by the ninth month. The Table summarizes spot field surveys before and 3 years after control in 16 districts, recording prevalence and density rates for (a) microfilariae in blood surveys and (b) larvae of *W. bancrofti* in mosquito surveys. The marked reductions «after» demonstrate the effectiveness of the diethylcarbamazine program. The potential transmission index dropped from 100 before control to an average of 3.2 afterward.

No new cases of hydrocele or of elephantiasis were reported in areas where control was in progress for a period of 2 years or longer. Attacks of primary filariasis also became minimal.

Prevalence in early age groups. — In evaluating the effectiveness of a filariasis control program the prevalence rates before and after control in the age group 0-4 provide significant information. It is the earliest group in which transmission can be evaluated accurately as it is the first group of originally negative persons who with certainty has not received diethylcarbamazine. Before control measures began, 926 children occurred in this age group. Fifty-two or 5.6 percent were positive for microfilariae, with a density of 30 per positive child. In the annual 1957 survey in Tahiti, 1,001 who had received no diethylcarbamazine occurred in this age group and all were negative. During the first half of 1958, however, 2 positive children have been detected in this age group. Both children lived in homes with positive parents who had refused to take the diethylcarbamazine. The percentage positive in the 0-9 age group dropped from 11.4 before to 0.6 after treatment. These findings show that new infections have been reduced to an extremely low level.

It has been shown by McGregor and Gilles (1956), Kessel (1957a) and others that a few carriers who were treated and became negative, then received no diethylcarbamazine, returned to a positive status after varying lengths of time. Follow-up examination in Tahiti have shown that 2.5 percent reverted to a positive state after 1 year and 6 percent after 3 years. The average number of microfilariae per 20 mm³ per positive in these groups was 6. It has likewise been

observed in the 1957 blood survey that 1.3 percent of previously negative persons yielded positive tests, with a microfilarial density of 7 per positive. These may be explained as (1) erroneous primary diagnosis because of a low count, (2) infections that were immature when first examined but which subsequently matured or (3) new infections. They are designated as «new infections» in our routine reports. McGregor and Gilles reported 21.5 percent recurrence after treatment, while combined recurrent and new infections in Tahiti amounted to 7.5 percent. It is possible that the different dosage of diethylcarbamazine used in Tahiti is responsible for the lower percentages obtained.

Because of the occurrence of such positive individuals in a population, either routine annual or biennial blood surveys are recommended in a control program, and the positives are again treated with diethylcarbamazine.

Longevity. — The longevity of filarial infections has been observed in 32 individuals, whose microfilarial counts were positive in 1949, and who received no diethylcarbamazine. Since then they have resided in areas of reduced transmission. Upon re-examination 7 to 8 years later, 11 or 34 percent were negative and 19 or 60 percent showed decreased counts. Two showed increased counts, which probably represented young infections in 1949. In 1957 a former resident of Tahiti returned from France, where he had resided 7 years. Examination showed him to have 4 microfilariae per 20 mm³ of blood. These results in general confirm the observations of Jachowski *et al.* (1950) that positive individuals exposed to little or no subsequent infection of *W. bancrofti* tend to become negative for microfilariae in from 5 to 10 years.

However, when contrasted with populations treated with diethylcarbamazine as in the Table, it is observed that a population unexposed to reinfection, but receiving no microfilaricide, requires a much longer time to become negative, and thus cease to be a transmission hazard, than a population which receives a microfilaricide.

(c) *Mosquito control.* — Because filarial infection in man, if untreated, persists for years, «mosquito control» alone, as a means of eradicating filariasis, would be a slow procedure even where methods used against adult and larval mosquitoes are sufficiently effective to approach their extermination.

Most investigators in the South Pacific agree that extermination of *A. polynesiensis*, which exhibits a wide variety of breeding places and seldom rests in houses, is expensive, and not practical.

A control program of eliminating mosquito-breeding places within 100 m of each dwelling was instituted early in the current program as a correlative measure with drug control, and has resulted in reducing the island-wide prevalence rate of adult mosquitoes to 0.2, or about one third of the original rate. Whether such mosquito reduction, alone, would be sufficient adequately to control filariasis in a short time is doubtful.

About 1950 the Cook Islands Medical Department organized a mosquito control program in Rarotonga which it was hoped would be helpful in reducing clinical filariasis. Six years later, Iyengar (1957) reported a microfilaraemia in Rarotonga of 23.3 percent which is similar to that previously reported by Amos in 1946. (See Faine and Hercus, 1951.) In contrast, Simpson (1957) reports a program which combines mosquito control and mass treatment with diethylcarbamazine on the Island of Niue. The prevalence of microfilaraemia dropped from 22.1 percent in 1954 to 2.9 percent in 1956. The former experience illustrates the disappointment that is common in employing mosquito control alone, but the latter demonstrates the rapid drop in filariasis prevalence when the combined method is used.

Once a satisfactory reduction in microfilarial prevalence is obtained by an adequate diethylcarbamazine campaign, it is possible that thorough mosquito control alone will maintain transmission at that low level where clinical filariasis will no longer be a problem.

CONCLUSIONS AND SUMMARY

1. This symposium reviews the latest knowledge concerning epidemiology and control of filariasis in Africa, Asia, the Americas and the Pacific.

2. This paper reports work from French Polynesia, where an extensive program has been in progress for some years.

3. Preliminary clinical surveys showed: elephantiasis, 14 percent; hydrocele, 16 percent; and lymphangitis, 27 percent to occur in males over 20 years of age, therefore presenting an important health and economic problem.

4. Preliminary blood examination and mosquito dissections showed: microfilaraemia, 32 percent; microfilarial density in 20 mm³ of blood for whole popula-

tion, 30; *A. polynesiensis* harboring all stage larvae, 11.4 percent, and infective stage larvae, 5 percent.

5. Of 8 species of mosquitoes, *A. polynesiensis* is most frequently infected in nature and is considered the major vector.

6. The filariasis control program combines:

(a) Mass administration to the whole population of diethylcarbamazine, once a month for 12 months in a single dose of 6 mg per kilogram of body weight. After a rest of 6 months, a blood survey is performed and positives continue treatment until negative.

(b) Mosquito control by eradicating breeding places within 100 m of each dwelling. This has decreased the adult mosquito population by about two thirds. Such mosquito control alone is considered inadequate for rapid filariasis control.

7. By the current program, microfilaraemia was quickly reduced and after the third year averaged 3.2 percent with a microfilarial density of 0.6. Only 0.3 percent of the mosquitoes harbored infective stage larvae. Transmission and new cases of clinical filariasis were likewise reduced to a minimum.

8. Before control 5.6 percent of the population in the age group 0-4 exhibited microfilariae. In 1957 all children in this age group were negative.

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A BRIEF REVIEW OF THE EPIDEMIOLOGY OF FILARIASIS IN THE SOUTH PACIFIC

M. O. T. IYENGAR

In the epidemiology of filariasis in the South Pacific, the interplay of variable factors such as the type of filarial infection, the mosquito vector, and the contact of the vector with the human host has produced a varied picture of the prevalence of filarial infection and of filarial disease in different parts of the region, which at first sight might appear discordant. Two races of *Wuchereria bancrofti* occur in this region: the nocturnal periodic race transmitted by night-biting mosquitoes occurs in the north and west; the non-periodic race transmitted by day-biting mosquitoes occurs in the east and southwest. Several species of mosquitoes, differing widely in their bionomics and breeding habits, are the vectors in the different areas: *Culex fatigans* in the northern zone, *Anopheles farauti* in the west, *Aedes polynesiensis* and allied species in the east, and *Aedes vigilax* in the southwest. Depending on the facilities available for breeding and the proximity of human habitations to breeding sites, the vector density and the extent of contact of the vector with the human host vary from locality to locality. How these factors have contributed to the diverse picture in the 4 zones distinguishable on epidemiological features is briefly discussed in this paper.

I. Zone A, with nocturnal periodic *W. bancrofti* transmitted by *Culex fatigans* comprises the Micronesian area of the Marianas, Carolines, Marshalls, Gilberts, Ocean Island and Nauru. The islands are small, lowlying and sparsely populated. The general incidence of microfilarial infection is low. In the more populated islands like

the Palau, Truk, and Wolei groups (Carolines), Saipan (Marianas), Tarawa (Gilberts) and Nauru, microfilaria rates are high (13 percent or over). Although cases of elephantiasis occur in most areas with high microfilaria rates, the incidence of elephantiasis is very low, less than 1 percent. Only in Nauru, where quasi-urban conditions prevail, is the elephantiasis rate higher (1.4 percent).

Culex fatigans, the primary vector in this zone, occurs on all the inhabited islands. Its prevalence is related to density of population as its preferred breeding places are collections of polluted water in the vicinity of habitations. On the sparsely populated islands the incidence of *C. fatigans* is low and microfilarial infection is absent. The more populated islands have a greater prevalence of *C. fatigans*; in these microfilariasis is present, the microfilaria rates varying in the different islands. Although in these islands the prevalence of *C. fatigans* is sufficiently high to maintain endemic filariasis, it is not high enough to establish hyperfilarisation and high elephantiasis rates. Conditions in these rural areas as regards availability of sullage water collections do not approximate those in urban areas of Southeast Asia.

II. Zone B, with nocturnal periodic *W. bancrofti* transmitted primarily by *Anopheles farauti* includes the Melanesian islands of New Guinea, Bismarck Archipelago, Solomon Islands and New Hebrides. Most of these are large islands with a mountainous interior. Filariasis is endemic in the flat coastal tracts and in certain lowlying inland areas along rivers and near marshes. In many of the endemic areas the microfilaria rates are high (15 to 30 percent). Manifestations of filarial disease are by no means uncommon; elephantiasis rates (taking all ages into consideration) range from 1 to 4 percent. In the coastal areas, *Anopheles farauti* is the principal vector; in a few isolated foci in the interior, other species transmit the infection — *Anopheles bancrofti* in Tanah-Merah; *Culex bitaeniorhynchus*, *C. annulirostris* and *Taeniorhynchus uniformis* in the Berau region. *Culex fatigans* is not a vector of importance in this zone.

Anopheles farauti being also the principal vector of malaria, filariasis often occurs co-endemically with malaria. Indiscriminate clearing of virgin jungle resulting in exposure of marshy areas to sunshine, and proximity of habitations to intensive breeding sites of

the vector are the main factors that favour the establishment of endemic filariasis.

III. Zone C, with non-periodic *W. bancrofti* transmitted by species of the *Aedes* (*Stegomyia*) *scutellaris* subgroup, includes Fiji, Ellice Islands and all Polynesian islands on the east. Filariasis is widespread in the numerous small islands of this zone. Its distribution is rural; urban areas are free from endemic filariasis. Microfilaria rates are 20 percent or more. Manifestations of filarial disease are of common occurrence, the elephantiasis rates ranging from 1 to 5 percent, sometimes higher. In the Wallis Islands and the Marquesas the incidence of elephantiasis is very high; on the other hand, in the Tokelau group, Tuamotu Archipelago and Austral Islands, microfilarial infection is present but elephantiasis is rare.

Five closely related species transmit the infection in different parts of this zone — *Aedes polynesiensis* and *A. pseudoscutellaris* in Fiji; *A. tongae* in Tonga; an undescribed species allied to *A. tongae* in Niue; a species allied to *A. polynesiensis* in Rotuma; and *A. polynesiensis* in other areas. All these vectors are day-biters, breed in artificial containers, and have a limited range of flight. Filarial endemicity is directly related to the density of the vector in and around habitations. Vector density depends on the availability of suitable breeding sites. The main breeding places are: (1) domestic containers for storing rainwater, necessitated by the absence of piped-water supply in most areas; (2) coconut-shells and discarded tin-cans — the people use a lot of coconuts and tinned meat for food, and the copra industry accounts for a large accumulations of coconut-shells; and (3) rat-damaged coconuts — on many islands rats destroy 30 to 50 percent of the coconut crop.

Although *Aedes* mosquitoes occur in bush as well as near habitations, the epidemiologically significant sites of transmission are those where close contact exists between the vector and the human host. It is only in and around villages that maximum opportunities are offered for the vector to derive the infection from the human reservoir and subsequently to transmit it to man. Where such close contact is lacking as in bush, the vector in view of its limited range of flight either fails to get infected or is unable to transmit to man any fortuitously acquired infection that may have matured. Transmission may

sometimes occur in areas away from habitations, but the vast majority of infections are contracted in the vicinity of habitations where also occur the series of re-infections necessary to step up the infestation to the level at which filarial disease manifests itself.

IV. Zone D, with non-periodic *W. bancrofti* transmitted by *Aedes (Ochlerotatus) vigilax*, covers a small area — the Loyalty Islands and New Caledonia. The vector being a salt-marsh breeder, endemic filariasis is restricted to a few small foci along the coast in the neighbourhood of mangrove swamps. Villages in the interior as well as coastal villages away from mangrove swamps are free from filarial infection. In the different endemic foci the microfilaria rates range from 1 to 37 percent. A notable feature is that cases of elephantiasis are rare, though cases of hydrocele occur in some foci. In a very small number of the foci elephantiasis rates are about 1 percent; in others elephantiasis is absent.

The rarity of cases of elephantiasis in the endemic foci is due to conditions being unfavourable for the establishment of hyperfilaria in the human host. Because of the distance (alf to one mile or more) of the breeding sites from the endemic foci, there occurs considerable scatter of infected mosquitoes during their gonotrophic movements. Although many of the infected mosquitoes return to the endemic foci, evidenced by their presence there and occurrence of microfilaraemia in the population, their number is not sufficiently large to facilitate intensive transmission and repeated reinfections necessary for the establishment of hyperfilaria in.

Conditions were very different in New Caledonia a hundred years ago, when a considerable part of the population lived in coastal villages close to mangrove swamps. The proximity of the habitations to extensive breeding sites of the vector favoured the establishment of hyperfilaria, and elephantiasis of the leg and scrotum was then of common occurrence. Following the native insurrection of 1878, the coastal populations were moved into the interior, while some were resettled in selected coastal areas near missions and police stations. Ruins of abandoned villages along the coast confirm this historical fact. The exodus to the interior cut off contact with the vector and, in consequence, the infection has died out in such communities. The resettled coastal villages were on new sites often far from mangrove

swamps; only those are endemic foci today as are within a mile of mangrove swamps. In these we have microfilarial infection but, due to lack of facilities for hyperfilariation, elephantiasis is rare.

In the South Pacific, the distribution of microfilaraemia covers a larger area than that of filarial disease. In several endemic areas, even in those with appreciable microfilaria rates, elephantiasis is absent or its incidence very low. In such areas, although transmission goes on, it is not intensive enough to produce hyperfilariation in the human host, a factor essential for the onset of filarial disease. Hyperfilariation could only happen where a high vector density prevails in close proximity to habitations.

From the public health standpoint, a significant prevalence of filarial disease is the important consideration; the mere presence of the vector or of microfilarial infection, in the absence of manifestations of filarial disease in the community, is of little significance. Considering the fact that a high vector density in close contact with the human host is necessary for the establishment of hyperfilariation, control of filarial disease in areas where it is of public health importance resolves itself to the control of the vector in and around inhabited areas so as to prevent hyperfilariation rather than to the extermination of the vector or the eradication of microfilarial infection. Neither of these two latter procedures, apart from the consideration whether they are practicable or even necessary, would be within the financial resources of the communities of the underdeveloped areas where filariasis is a public health problem.

FILARIASIS IN MALAYA

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INTRODUCTION

This article reviews the knowledge available about filariasis in Malaya as at 31st March 1958. Investigations by Institute staff are still in progress, and the views expressed are liable to future modification.

METHODS AND STANDARDS

Our criteria for the diagnosis of filariasis have been the finding of microfilariae in blood films and/or the presence of a definite elephantiasis.

Blood films were collected after nightfall by finger-prick and capillary tube, usually between 19:00 hours and midnight. For the Pahang surveys and some of the Penang ones, the tubes were calibrated to 20 mm³; elsewhere the films approximated to this amount. Films were dried overnight, stained with Giemsa in phosphate buffer solution, and the microfilariae counted.

A house-to-house census was made before each survey, and the people were examined by households to ensure that all age-groups were represented. In Pahang, the surveys covered 90 percent of the population in the selected areas.

THE PARASITES

General. — In the Federation of Malaya, the predominant species is *Wuchereria malayi*. Two small endemic foci of periodic *W. bancrofti* have recently been found, otherwise infections with this species

are usually imported from India or China. A third species of *Wuchereria*, *W. pahangi*, occurs in animals (Buckley and Edeson, 1956), but has not so far been found in man.



Fig. 1 — Distribution of filariasis in Malaya

The scattered rural distribution of the endemic areas is shown in Fig. 1. The Malays are the main sufferers, because they form the bulk of the population in the rural areas concerned.

Two forms of «*W. malayi*».—Earlier investigations served to define the main endemic foci, but few patients received detailed examination, and differences between them were regarded as individual variations. We soon recognised that endemic filariasis was

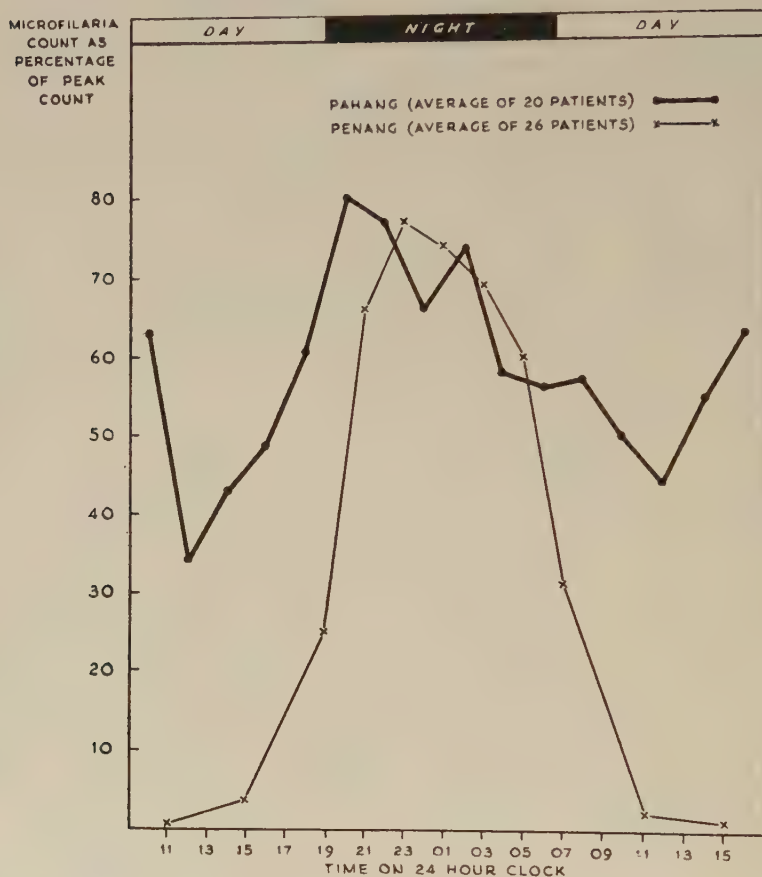


Fig. 2 — Periodicity of microfilariae of *W. malayi* in Malaya (after Turner and Edeson, 1957)

associated with two main types of terrain, each with its characteristic mosquito fauna, but were much slower in realising that there were consistent differences in the behaviour of the microfilariae to be found in each type of terrain. The evidence now seems sufficient to

warrant the statement that two forms of *W. malayi* exist in Malaya; for the present, these are named for the areas in which they have been found.

One of the main differences between the forms is the degree of nocturnal periodicity, illustrated in Fig. 2.

In Kedah/Penang, the microfilariae show marked nocturnal periodicity, and are rarely found in daytime blood films. Empty sheaths are common in thick films, and many microfilariae have wholly or partly emerged from their sheath; the mean length of formalin-fixed microfilariae is greater than those from Pahang. In Pahang, there is some nocturnal rise in microfilaria count, but microfilariae can be found readily at any time. Empty sheaths are very rarely seen, and many microfilariae are still ensheathed. There are also differences connected with the vector mosquitoes which will be described later.

Although we have not observed differences in the clinical manifestations of filariasis, we consider it desirable to present separately the data for the areas in which the two forms are found.

THE HUMAN HOST

Infection rates.— Figs. 3 and 4 illustrate the trend of infection rates by sex and age-group.

The main difference is one of degree. In Pahang, infection rates rise more steeply and to a higher level; this is thought to be due to a greater intensity of transmission. There is little difference in mean microfilaria counts: this figure for 20 mm³ in whole population was 16.1 in Kedah/Penang, and 18.5 in Pahang. We cannot explain the tendency for women in Pahang to show lower total infection rates than the men, whereas little difference is found in Kedah/Penang. Apart from this trend in Pahang, peak infection rates are reached in childhood, with only minor variations thereafter; a tendency for microfilaria rates to fall in adult life is counter-balanced by the increase in elephantiasis from 10 years onwards. Most patients with elephantiasis do not have microfilariae in their blood, thus the total infection rate remains fairly steady. Considering that the microfilaria

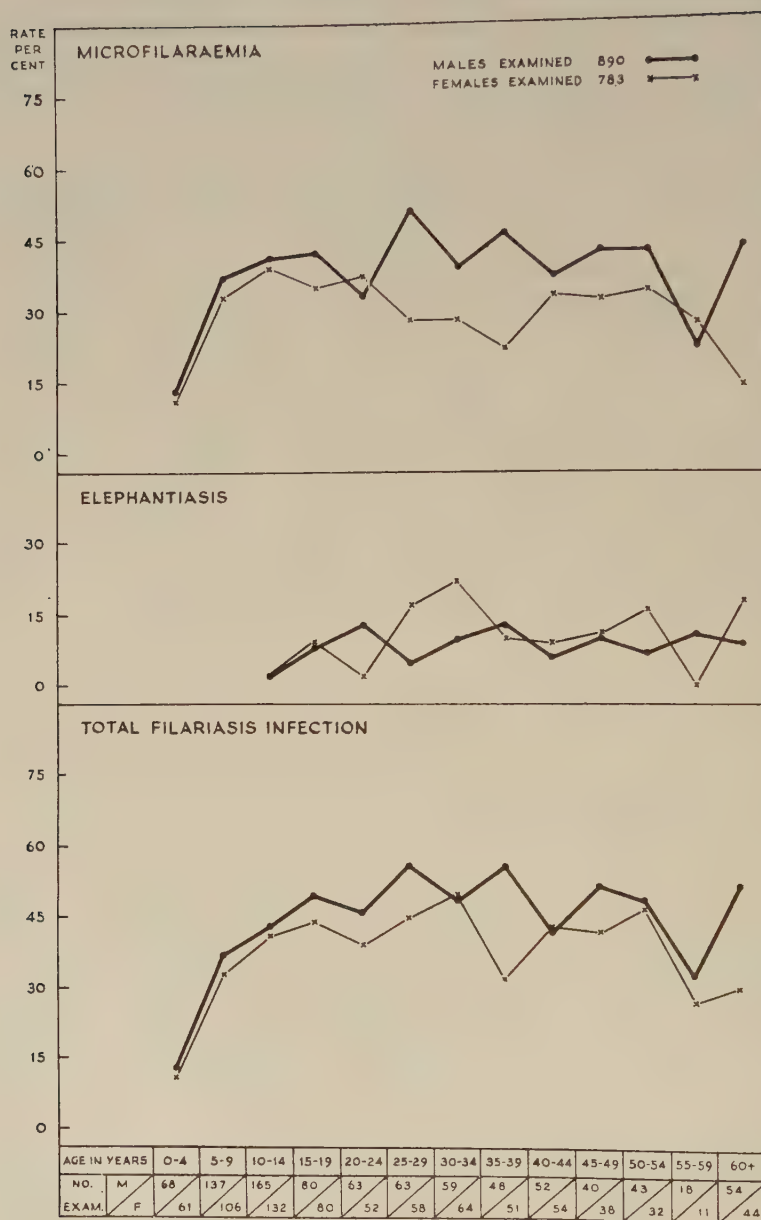


Fig. 3 — Kedah/Penang: filariasis infection rates by age and sex

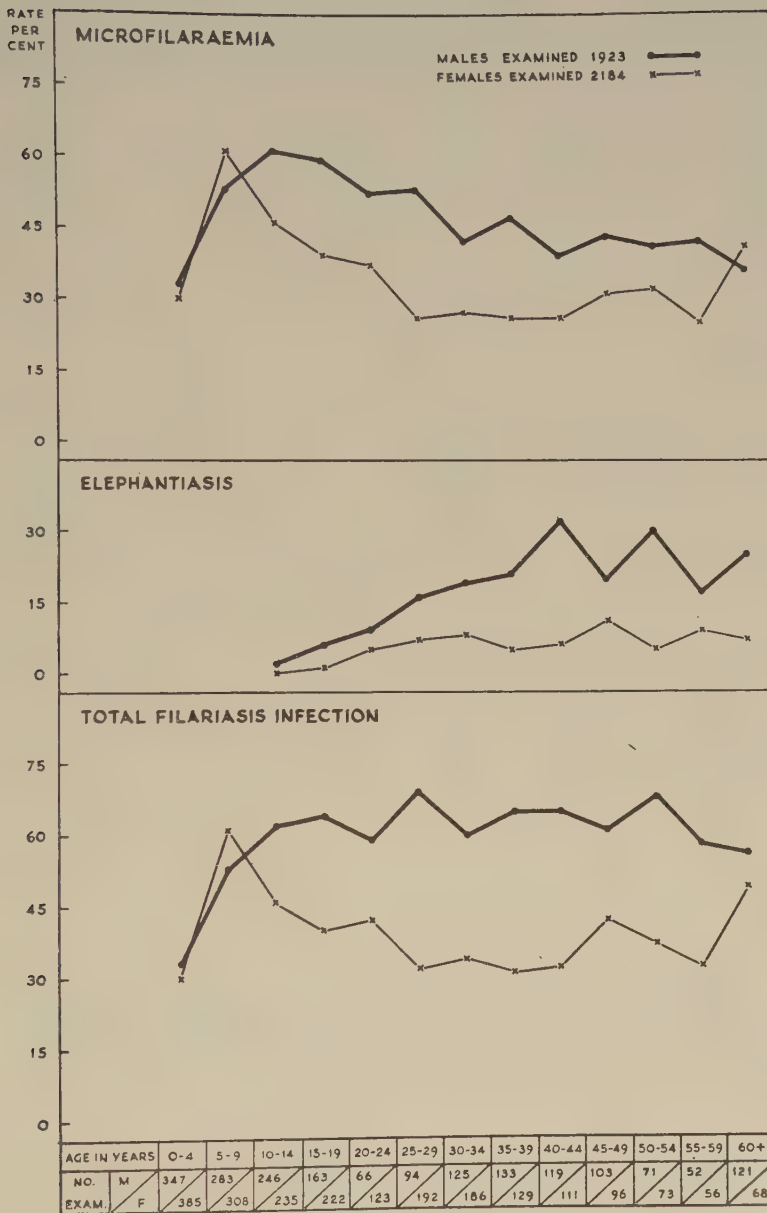


Fig. 4 — Pahang: filariasis infection rates by age and sex

rates are based on a single examination of a 20 mm³ blood film, it seems probable that—at least in Pahang—the true total infection rate must be close to 100 percent from about 5 years of age onwards.



Fig. 5 — Microfilaraemia in children, 0-14 years

Children are infected very early in life, as shown in Fig. 5.

In Pahang, the youngest child found infected was aged 3½ months; at age 4-5 years the microfilaria rate was 57 percent.

In Kedah/Penang, the youngest infected child was 13 months, and the microfilaria rate rose more slowly to 45 percent at 9-10 years. Despite this apparent difference in intensity of transmission, the youngest child seen with elephantiasis in each area was aged 10 years.

Clinical filariasis.—The clinical features attributable to filarial infection are similar in the two areas. The early stages of infection seem symptomless; most microfilaria carriers are unaware of their condition, and many have no detectable glandular enlargement or other clinical sign of infection. Diagnosis at this stage must depend upon the finding of microfilariae in the blood.

The first intimation of infection to the patient is an attack of fever accompanied by adenolymphangitis. Turner (1958) has termed these attacks «episodic» rather than «periodic», because they recur at irregular intervals. The proportion of the population reporting these attacks ranges from 6 to 15 percent. The episode may be accompanied by some swelling of the affected limb: this is at first temporary and subsides completely between attacks, but later becomes a permanent elephantiasis.

The temporary swelling, and the subsequent elephantiasis, almost always affect one or both legs. Fig. 6 gives details of the relative frequency of the limbs affected in 387 patients with elephantiasis.

In more than half of the patients, the elephantiasis affected both legs: in the remainder, there was a slight tendency for the right leg to be more often affected than the left. Involvement of the arms was rare, and only one patient with scrotal elephantiasis has been seen.

DRUG TREATMENT

Diethylcarbamazine is the drug of choice. Wilson (1950) showed that hetrazan was perhaps even more effective against the microfilariae of *W. malayi* (in Kedah) than against those of *bancrofti*; microfilariae remained absent for 2½ years or more after one course of treatment. A sharp febrile reaction to treatment occurred in microfilaria carriers. Subsequent drug trials have not revealed any method of avoiding this reaction. Doses given once daily for 14-17 days

were satisfactory in hospital, but impracticable for mass outpatient treatment.

Then the report by Kessel *et al.* (1953) of the effect of spaced doses once a month led to similar trials here, with the results shown in Table 1.

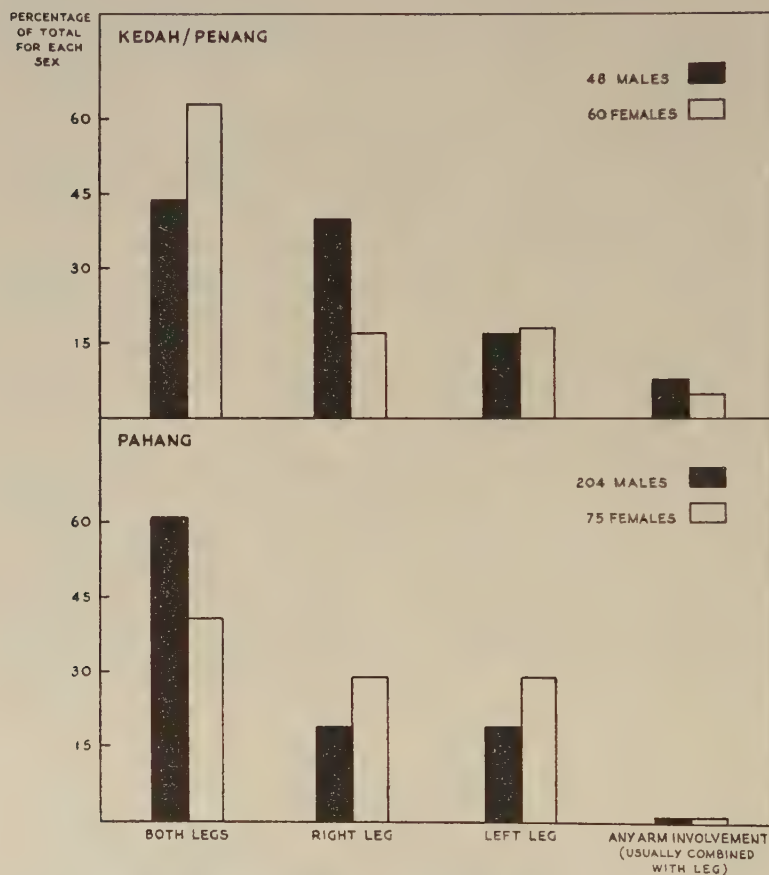


Fig. 6 — The affected limb in elephantiasis due to *W. malayi*

A dose of 4-6 mg/kg apparently was needed for microfilaria clearance. Similar results were obtained from doses given once a week, and these two schedules were later used for pilot control schemes in rural areas.

THE VECTORS

The two forms of *W. malayi* are associated with different types of terrain and hence with different mosquito faunae.

Kedah/Penang.—Endemic filiarisis occurs in coastal ricefields interspersed with patches of open swamp. Natural infections have

TABLE 1 — *The treatment of W. malayi microfilaria carriers with varying doses of diethylcarbamazine citrate given once a month for six months (after Edeson & Wharton, 1958a)*

Dose in milligrams per kilogram of body weight	Number treated	Number found positive (months after first dose)							Average microfilaria count per 60 cm ³ before treatment	Percentage reduction in microfilaria count (months after first dose)						
		1	2	3	4	5	6	12		1	2	3	4	5	6	12
1	12	11	11	12	11	10	10	7	281	52	75	87	84	93	93	98
2	12	12	11	10	8	8	5	2	186	55	88	94	97	96	98	99
4	11	11	11	5	5	4	4	0	123	58	93	98	99	99	99	100
6	13	13	9	7	6	3	2	0	286	88	96	99	99	99	99	100

been found in the *Anopheles hyrcanus* group, *A. barbirostris*, and *Mansonia indiana*, *uniformis* and *annulifera*. The natural infection rate is variable, and the proportion harbouring infective stage larvae averages 0.2 percent. Transmission probably almost ceases during a dry season of about 2 months each year.

Pahang.—The endemic areas lie along the banks of the Pahang river and are surrounded by many square miles of fresh water swamp forest (Fig. 7).

These swamps form extensive breeding places for *Mansonia longipalpis*, which occurs in large numbers, with *M. annulatus* and *M. uniformis* in more restricted distribution. All these have been found naturally infected; the proportion harbouring infective larvae averages 0.6 percent. The biting rate however is so high that a figure

of 0.2 infective bites per man-hour has been recorded on various occasions, and transmission occurs throughout the year. Anophelines are few in number, and unimportant.

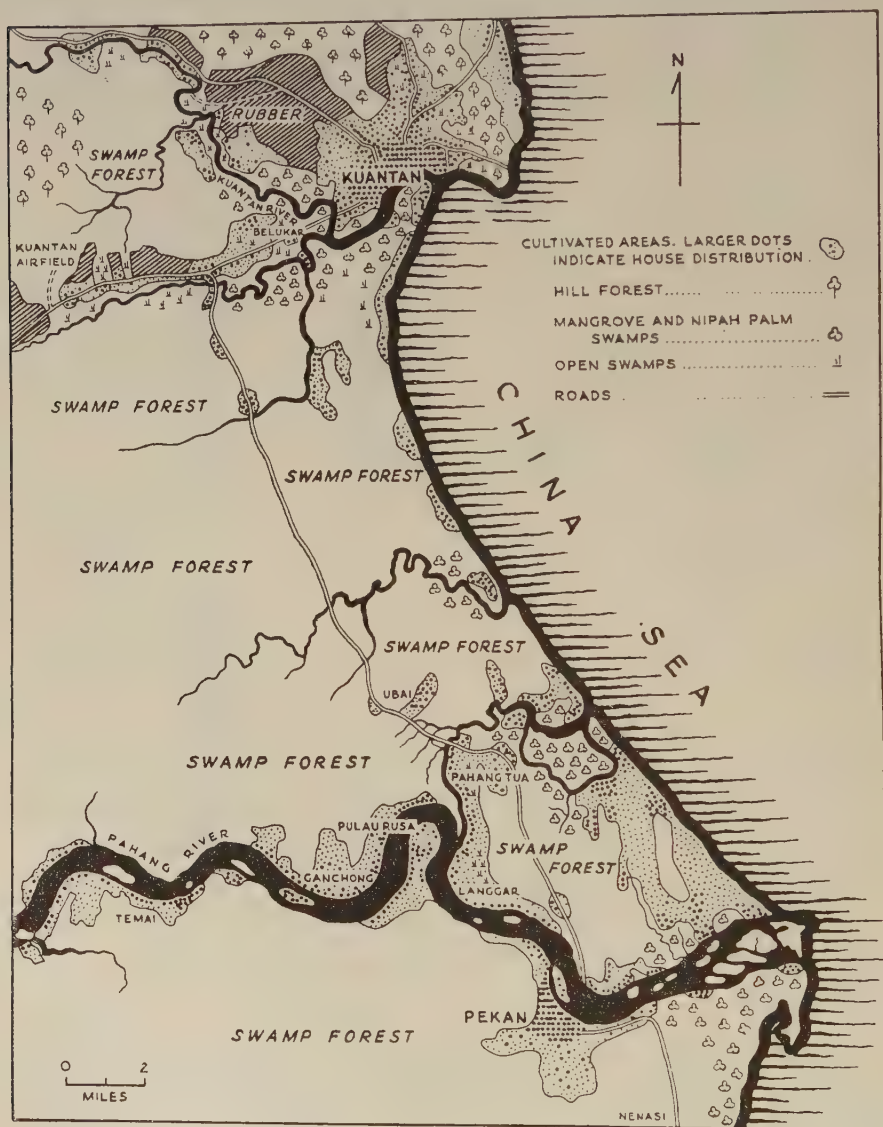


Fig. 7 — Map of East Pahang (after Wharton *et al.*, 1958)

The interpretation of mosquito infection rates in Pahang is rendered difficult by the presence of natural infections of two species of *Wuchereria* in animals, *W. malayi* and *W. pahangi* (Buckley and Edeson, 1956).

Table 2 is a list of animals found infected in Pahang; a search for such infections in the Kedah/Penang area has so far been limited to cats.

TABLE 2 — *Natural infections with Wuchereria species in domestic and forest animals in East Pahang, Malaya (as at 31 March 1958)*

Species	Number examined	Number harbouring <i>Malayi</i> -type microfilariae	Confirmed host for	
			<i>W. malayi</i>	<i>W. pahangi</i>
<i>Primates :</i>				
Long-tailed macaque	87	4	Yes	—
Leaf monkey	7	6	Yes	—
Slow loris	28	2	—	Yes
<i>Carnivores :</i>				
Domestic cat	159	50	Yes	Yes
Palm civet	29	7	—	Yes
Flat-headed wild cat	1	1	—	Yes
Leopard cat.	1	1	—	—
Tiger	1	1	—	Yes
Domestic dog	11	6	—	Yes
<i>Insectivores :</i>				
Moon rat	2	2	—	Yes
Pangolin	9	1	—	—
<i>Rodents :</i>				
Giant squirrel	2	1	—	—

Although these two species of *Wuchereria* differ in their infectivity to species of *Mansonia*, the larvae which do develop in the mosquito are indistinguishable, thus it is impossible to determine the source of the mosquito infections. This difficulty hampers our efforts to assess the results of control measures by mosquito dissections.

Laboratory experiments.—In artificial feeding experiments, *M. longipalpis* prove an extremely efficient host for *W. malayi* from Pahang, but a very poor host for *W. malayi* from Kedah.

In contrast, *A. barbirostris* could be readily infected with *W. malayi* from Kedah, but was a very poor host for *W. malayi* from Pahang.

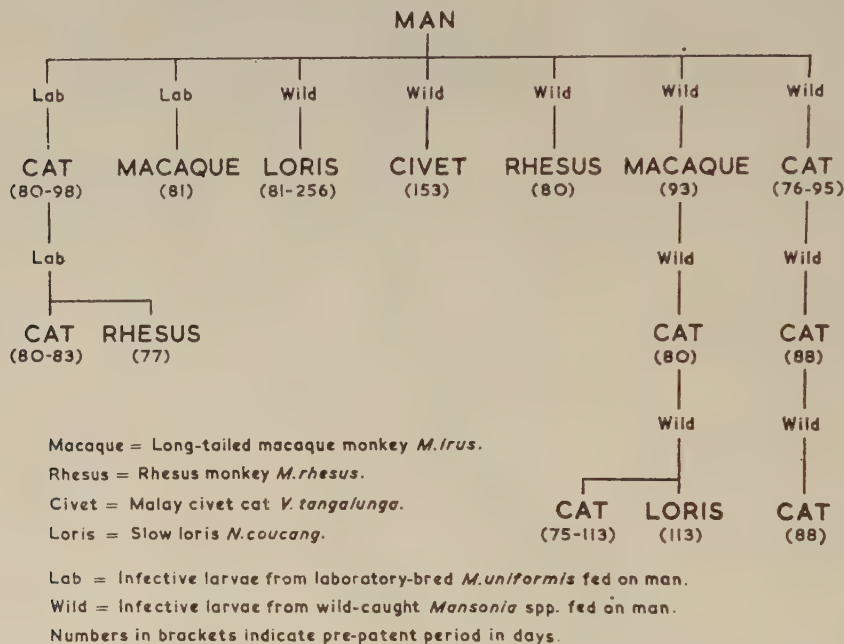


Fig. 8 — The experimental transmission of *W. malayi* from man to animals (after Edeson and Wharton, 1958b)

Other experiments have shown that the human *W. malayi* in Pahang can be transmitted to a variety of animals (Fig. 8).

The prepatent period of about 80 days in a suitable host corresponds closely to the age of the youngest child found with a natural infection (3 ½ months).

These transmissions were by syringe inoculation of infective larvae; more recently, transmission to cats by mosquito bite has also been accomplished. The reverse transmission from animals to man

may well occur in nature, thus impeding attempts at control of the disease.

CONTROL TRIALS

In two small settlements in Pahang, mass treatment with diethylcarbamazine was carried out (doses of 5 mg/kg of body weight once a month for 6 months, and once a week for 6 weeks); in a third, houses were sprayed with dieldrin, 100 mg/sq. foot every 6 months. The results of these control measures on microfilaria rate and density are shown in Fig. 9, and their effect on the infective larva rate in mosquitoes in Fig. 10.

Drug treatment produced the expected immediate reduction in microfilaria rate and density, but both figures have risen steadily since then; in the sprayed settlement, there has been a slight reduction after 3 years.

Preliminary trap-hut experiments, and mosquito catches in sprayed houses, indicated that the dieldrin remained effective against *M. longipalpis* for about 6 months. Despite this, and despite the great reduction of the human microfilarial reservoir in the drug-treated areas, neither method of control had any clear effect on the proportion of *M. longipalpis* harbouring infective stage larvae of the *W. malayi* type. One must conclude that some untouched reservoir of microfilariae exists, which is sufficient to maintain transmission.

PLANS FOR FUTURE CONTROL

Pahang.—In the hope of obtaining better results, control measures are being extended to larger areas, and a combination of drug treatment and residual spraying will be used. Mass treatment, as hitherto employed, was not completely effective: the decision to give 6 doses was based on the good results obtained in the hospital trials, and also because considerable difficulty was expected in getting the local people to take the later doses. This expectation was realised, and 10 to 12 visits were required to persuade 81 percent of the population to take even 6 doses. We now plan to resurvey in a year's time, and repeat the drug treatment of microfilaria carriers.

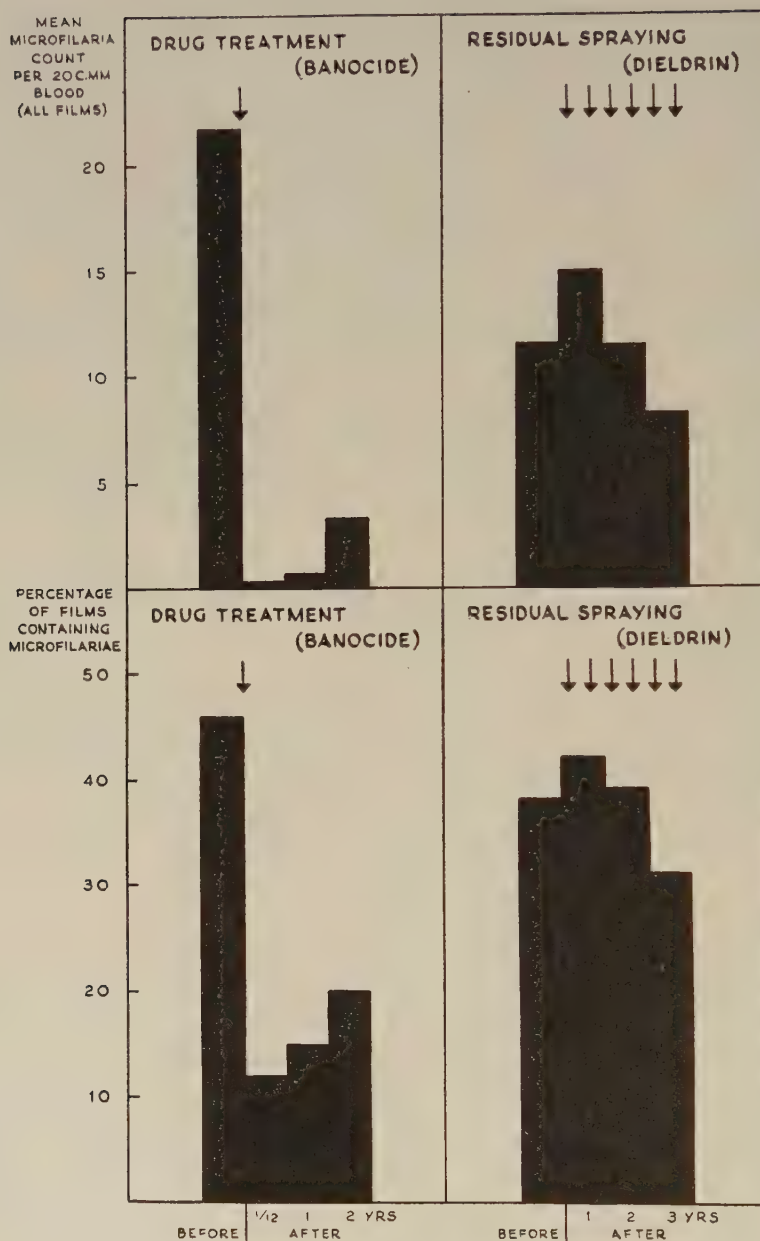


Fig. 9 — Effects of control measures (after Wharton *et al.*, 1958) on microfilaria density and microfilaria rate

Kedah. — A methodical control programme has been started by the State Health Department. Mass treatments and residual spraying are being used together, and follow-up surveys are planned.

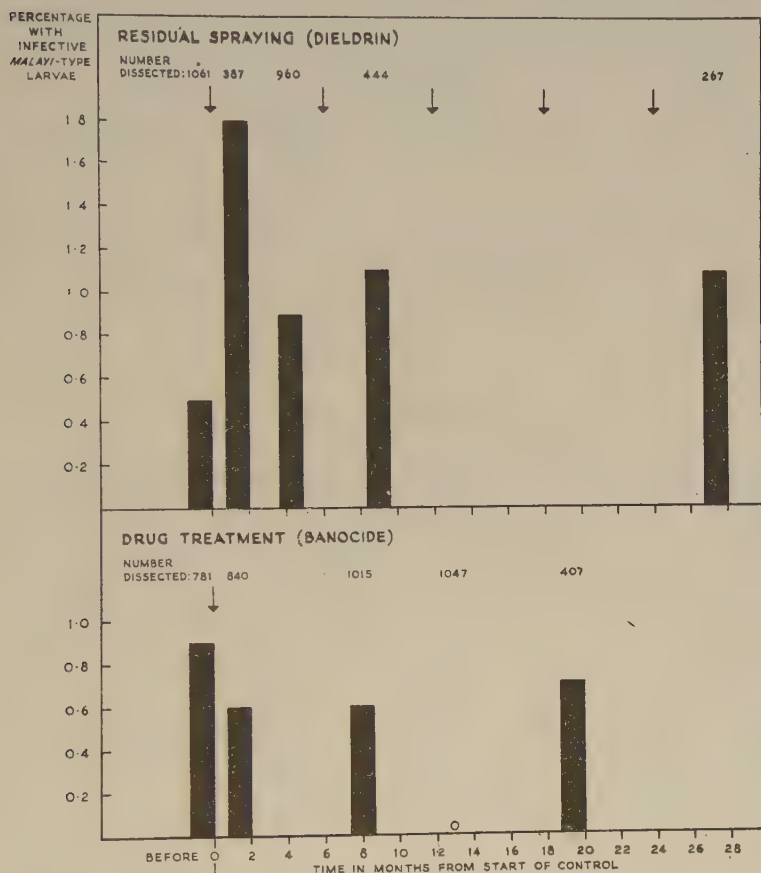


Fig. 10 — Effects of control measures (after Wharton *et al.*, 1958) on infection rate in *M. longipalpis* — mature *malayi*-type larvae only

A final control policy for Malaya has not yet been formulated; the recommendations to be made will depend upon the results obtained from the work now being done, and may well be different for the two main types of endemic area and the two forms of *W. malayi*.

SUMMARY

1. Filariasis in Malaya is mainly due to *Wuchereria malayi*, two forms of which occur; the differences are described.
2. Children are infected at a very early age, and a microfilaria rate of 57 percent at 4-5 years has been recorded.
3. Elephantiasis due to *W. malayi* is almost confined to the legs.
4. Diethylcarbamazine is an effective treatment.
5. The vectors in Kedah/Penang are anophelines and *Mansonia* other than *longipalpis*: in Pahang the vectors are *M. longipalpis*, *M. annulatus*, and *M. uniformis*.
6. In Pahang, natural infections of *W. malayi*, and a new species *W. pahangi*, occur in a number of animals. Various species of animals have been experimentally infected with human *W. malayi*.
7. Pilot control trials indicate that interruption of transmission will be difficult.
8. Future control policy has not yet been formulated.

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FILARIASIS IN INDONESIA; DISTRIBUTION, INCIDENCE AND VECTORS

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and

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This report contains a summary of published information and the research now in progress on filariasis in Indonesia.

The known distribution and incidence of *Wuchereria malayi*, periodic *W. bancrofti*, and elephantiasis are recorded on the accompanying map. The map also shows areas in which *Wuchereria* and/or elephantiasis have been reported in public health records and other accounts, but the species, incidence and extent of the areas were not included. The designated negative results were obtained from limited samplings taken in these areas and do not exclude the probability of locating filariasis in these areas by more extensive surveys.

It is evident from the data contained on the map that the studies to date have been conducted in more accessible places along the coasts and inland, on major rivers and roads. Existing information indicates that filariasis probably will be found in many of the areas from which information on this subject is not available at present.

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A summary of human blood surveys recently conducted in Bengkulu, South Sumatra, and in Mampang-Perapatan District, Djakarta, is presented in Table 1.

TABLE 1 — *Summary of a blood survey in Mampang-Perapatan District, Djakarta, and Bengkulu, Sumatra*

Age	Sex	Djakarta. <i>W. bancrofti</i>		Bengkulu. <i>W. malayi</i>	
		Number examined	Percentage positive	Number examined	Percentage positive
1	M	7	0	9	0
	F	8	0	16	0
1-5	M	220	4.5	510	14.1
	F	195	5.6	536	21.5
6-10	M	232	19.2	425	33.7
	F	222	17.6	319	37.9
11-20	M	725	25.2	784	39.7
	F	607	28.8	697	45.3
21-30	M	677	28.1	685	48.6
	F	619	23.3	607	36.9
31-40	M	579	27.3	593	48.6
	F	320	22.8	491	42.2
41-50	M	304	29.3	310	53.6
	F	138	23.2	272	48.5
50	M	158	23.4	189	46.6
	F	80	33.7	165	49.1
All ages	M	2,952	24.4	3,505	40.0
	F	2,189	22.9	3,103	38.5
Total	M + F	5,141	23.8	6,608	39.3
Average microfilaria / 20 mm ³			31.1	—	42.0

Available information concerning the known vectors of *Wuchereria* in Indonesia is presented in Tables 2 and 3. Included in these tables are the localities in which studies have been made, the references

TABLE 2 — *Reported vectors of W. malayi in Indonesia*

Locality	Reference	Vectors	Experimental infection rate	Natural infection rate
1. Bengkulu South Sumatra	Brug and de Rook, 1930	<i>M. longipalpis</i> <i>M. annulata</i>	93 % (109) 83 % (178)	1.2 % (323) 1.9 % (339)
2. Bengkulu South Sumatra	Rees <i>et al.</i> , 1958	<i>M. annulata</i>	—	1.4 % (699) 0.4 % inf. stages
3. Mamudju Central Celebes	Jurgens, 1932	<i>A. barbirostris</i> (probably <i>A. vanus</i>) <i>M. longipalpis</i>	83 % (108) 64 % (44)	8.9 % (214) 2.7 % (75)
4. Kalawara Central Celebes	Brug, 1937	<i>A. barbirostris</i> (probably <i>A. vanus</i>)	99 % (95)	8.1 % (271) 3.1 % inf. stages
5. Island of Ceram . . Moluccas	Brug and de Rook, 1933	<i>M. uniformis</i> <i>A. bancrofti</i>	— —	— —
6. Delta of Seraju . . Java	Rodenwaldt, 1934	<i>M. uniformis</i> <i>M. indiana</i> <i>A. «hyrcanus»</i> (= group)	66.6 % (12) 71 4 % (7) 88.9 % (18)	— — —
7. Bintjau Kalimantan	Kariadi, 1938	<i>M. annulifera</i> <i>M. uniformis</i> <i>A. barbirostris typicus</i>	93.9 % (16) 100 % (7) 100 % (7) 484 microf./ 20 mm ³	21.7 % (176) 5.9 % (101) 6.9 % (29)
8. Martapura Kalimantan	Kariadi, 1941	<i>A. venhuisi</i>	70 % (167) 472 microf./ 20 mm ³	7.0 % (243)
9. Upper Mahakam . . Kalimantan	Oey Djoe Hoat, 1942	<i>M. longipalpis</i> <i>M. annulata</i> <i>M. indiana</i> <i>M. crassipes</i>	76 % (46) 70 % (34) 74 % (99) 26 % (80)	— — — —

TABLE 3 — *Reported vectors of W. bancrofti in Indonesia*

Locality	Reference	Vectors	Experimental infection rate	Natural infection rate
1. Djakarta	Brug, 1920	<i>C. fatigans</i> (= <i>quinquefasciatus</i>)	59 % (22)	—
2. Djakarta Kramat-Salemba	Flu, 1929	<i>C. fatigans</i>	—	6 %
3. Djakarta	Suwadji Prawirohardjo, 1939	<i>C. fatigans</i> <i>A. sundicus</i> <i>A. subpictus</i> <i>A. fuliginosus</i> (= <i>annularis</i>) <i>A. vagus</i> <i>A. barbirostris typicus</i> <i>A. tessalatus</i>	61 % (102) 50 % (18) 72 % (54) 66 % (6) 55 % (9) 66 % (6) 100 % (3)	— — — — — — —
4. Djakarta Rawasari District	Chow <i>et al.</i> , 1958	<i>C. fatigans</i>	—	1.8 % (24,271) 0.3 % inf. stages
5. Djakarta Mampang-Perapatan District	Lie Kian Joe <i>et al.</i> , 1958	<i>C. fatigans</i>	—	7 % (25,394) 1.0 % inf. stages
6. Island of Kabaena	Brug, 1938	<i>A. aconitus</i> <i>C. fuscocephalus</i> <i>C. fatigans</i> <i>A. maculatus</i> <i>C. whitmorei</i> <i>A. barbirostris typicus</i> <i>C. annulirostris</i> <i>C. vishnui-alis</i> (= group)	68 % (31) 75 % (12) 33 % (175) + (1) 100 % (3) 29 % (144) 66 % (3) 45 % (197)	— — — — — — — —
7. Upper Digul . . . W. Irian	Elsbach, 1937	<i>A. bancrofti</i>	49 % (284)	10.9 % (655)

Locality	Reference	Vectors	Experimental infection rate	Natural infection rate
8. Hollandia W. Irian	Toffaletti and King, 1947	<i>A. farauti</i>	—	46.2 % (13)
		<i>A. punctulatus</i>	—	8.3 % (36)
		(intermediate form)	—	7.1 % (154)
		<i>Armigeres obturbans</i>	—	2.2 % (268)
9. Sorong W. Irian	de Rook, 1947	<i>A. farauti</i>	82 % (61)	—
10. Hollandia W. Irian	Metselaar, 1955	<i>A. karwari</i>	—	—
11. Island of Pam . .	de Rook, 1957	<i>A. farauti</i>	92 % (50)	41.1 % (467) 6.6 % inf. stages
		<i>Aedes kochi</i> (= group) (95 microf./ 20 mm ³)	70 % (111) (95 microf./ 20 mm ³)	15.6 % (190) 1.6 % inf. stages
12. Berau Region . . W. Irian	de Rook, 1957	<i>C. annulirostris</i>	89.7 % (156)	25.5 % (184) 1.6 % inf. stages
		<i>M. uniformis</i>	94.2 % (97)	19.8 % (227) 1.3 % inf. stages
		<i>C. bilaeniorhynchus</i>	87 % (54) (24 and 104 microf./ 20 mm ³)	19.7 % (142) 2.1 % inf. stages
13. Bangi-Ia area . . W. Irian	van Dijk, 1958	<i>M. uniformis</i>	91 % (56)	—
		<i>M. papuensis</i>	83 % (43)	—

in which the information was obtained, the vectors reported and the experimental and natural infection rate of the vectors expressed in the percentage positive of the mosquitoes examined. The figures in parenthesis are the number of mosquitoes examined in making this determination. Undoubtedly the methods used by the different investigators varied considerably in the collecting and examination of the

material, and in the number of microfilariae per unit in the blood upon which mosquitoes were fed to determine the experimental infection rate. The data, therefore, are only indicative of the results of the studies of each investigator and are not statistically comparable for all of the reports.

CONCLUSIONS

Wuchereria malayi and periodic *W. bancrofti* are widely distributed in Indonesia with a relatively high incidence in man and frequently without visible clinical symptoms. Both species occur principally in costal regions and inland waterways.

W. malayi is generally distributed throughout western Indonesia and east to the island of Ceram. It is rural in occurrence and is frequently associated with elephantiasis of the lower extremities and recurrent lymphangitis.

W. bancrofti is prevalent in West Irian and appears more sporadically westward on the other islands. It is commonly associated with elephantiasis and hydrocele in West Irian and on the island of Buruh, but elephantiasis rarely appears with *W. bancrofti* in Djakarta and Sumba, where other extensive surveys have been conducted, although hydrocele is common.

Important mosquito vectors of filariasis in Indonesia are reported as follows: *W. malayi*: *Mansonia annulata*, *M. annulifera*, *M. longipalpis*, *M. uniformis* and *Anopheles barbirostris* (= probably *A. vanus*).

W. bancrofti: *Culex fatigans* is an important vector in Djakarta, but apparently it is not an important vector in eastern Indonesia. *Anopheles farauti*, *A. punctulatus*, *Aedes kochi* (group) and *C. bitaeniorhynchus* are reported as important vectors in West Irian. Other vectors will undoubtedly be discovered as the investigations continue.

Filariasis constitutes an important public health problem in Indonesia because of its high incidence and wide distribution. It is difficult to evaluate the disabling effects, but the losses caused by filariasis in the efficiency of labor, man work days, and in the care required for those suffering in advanced stages of filariasis and associated afflictions, create an enormous economic and social loss to people of Indonesia.

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EPIDEMIOLOGY AND CONTROL OF BANCROFTIAN FILARIASIS IN AFRICA, WITH PARTICULAR REFER- ENCE TO TANGANYIKA

DR. P. JORDAN

INTRODUCTION

Stoll (51) has calculated that 22 million people in Africa are infected with Bancroftian filariasis, and although it has been reported from nearly every country between the North African coast and Southern Rhodesia (2, 5, 7, 8, 10, 11, 19, 24, 30, 34, 36, 40, 41, 42, 43, 44, 49, 55), there are practically no extensive or detailed studies of the disease from this continent.

Owing to considerable differences in geography, climate, mode of life of the different peoples and numerous other factors, within the continent of Africa it cannot be claimed that data obtained from any one area will be applicable to other parts of the continent. It is proposed therefore to discuss Bancroftian filariasis as it is seen in East Africa and to refer in the appropriate place to reports of the disease from other territories.

DISTRIBUTION OF BANCROFTIAN FILARIASIS IN TANGANYIKA

In Tanganyika extensive investigations into the distribution and incidence of filariasis were made by taking blood samples at night from the populations of nearly 150 villages throughout the territory (24). Unmeasured samples of blood of approximately 20-30 mm³ in volume were taken.

Bancroftian infection was found in about a fifth of the territory—infection rates (in adult males) ranging from 0 percent to over 60 percent were found.

The infection was restricted to the coastal areas, river valleys, and other low lying areas, but a further focus near Lake Victoria, nearly 1,000 miles inland, is at an altitude of nearly 4,000 ft.

SEX INCIDENCE

As in many other parts of the world, a higher incidence of infection was found in males, but whereas elsewhere this has been accounted for by males being exposed more to infection than females (22), in Africa where the night biting *Culex fatigans* and *Anopheles gambiae* and *funestus* are the main vectors, both sexes are probably exposed to infection to the same extent.

AGE INCIDENCE

The incidence of infection increased in the older age groups. Children were found to be more frequently infected in hyperendemic areas than in areas of low endemicity, and a few instances of infection in children in the first year of life were found (24).

MICROFILARIAL DENSITY IN RELATION TO MICROFILARAEMIA RATES

Data from villages having similar microfilaraemia rates (in adult males) were combined and 5 groups with different levels of endemicity were thus obtained.

- Group I—Infection rates 0-5 percent (non-endemic?). It is possible that in villages in this group transmission does not occur, the infections found having been acquired elsewhere.
- Group II—Infection rates 6-15 percent (low endemic).
- Group III—Infection rates 16-30 percent (moderate endemic).
- Group IV—Infection rates 31-45 percent (high endemic).
- Group V—Infection rates 46 percent and above (hyperendemic).

The overall infection rate (in adult males), the mean microfilarial density (per positive case) and the incidence of genital filariasis (hydrocoele, scrotal elephantiasis and lymph scrotum) in these groups are shown in Table 1.

TABLE 1 — *Relationship in adult males between the microfilaraemia rate, mean microfilarial density and the incidence of genital filariasis in village groups having different levels of endemicity*¹

Endemic group	Microfilaraemia rate (percent)	Mean microfilarial density	Genital filariasis rate (percent)
I (1-5 percent).	3	3.3	1.6
II (6-15 percent).	9.7	6.9	5.7
III (16-30 percent).	21.9	11.7	13.1
IV (31-45 percent).	35.9	16.7	18.3
V (46 percent and more). .	53.4	25.5	31.1

¹ Data from villages in which no microfilariae of *W. bancrofti* were found have been excluded.

In Table 2 data from the 5 different groups are combined to show the variation in incidence of microfilaraemia, mean microfilarial den-

TABLE 2 — *Variation with age of the incidence of microfilaraemia, mean microfilarial density and incidence of genital filariasis in males*

	Age group							
	0-5	6-15	16-25	26-35	36-45	46-55	56-65	66 →
Numbers examined	585	2,833	1,783	2,515	2,311	1,521	571	181
Incidence microfilaraemia (percent).	1.5	6.8	17.8	22.9	25	27.6	29.9	28.1
Mean microfilarial density (per positive blood) . . .	5.2	8.7	12.0	14.9	17.8	16.9	17.8	16.5
Genital filariasis rate (percent)	0	0	3	9.6	13.6	19.7	23.3	22.5

sity and incidence of genital filariasis in relation to different age groups.

CLINICAL FILARIASIS

Acute filariasis, characterised by lymphadenitis, funiculitis and epididymo-orchitis, are occasionally seen in East Africa, but do not present the same problem as «Mumu» apparently does in the Pacific. They invariably resolve spontaneously, and are rarely seen in hospital practice. Such acute manifestations may also complicate chronic filarial conditions such as elephantiasis of the limbs, breast, vulva or scrotum, and the very much more prevalent hydrocoele. Less obvious, but perhaps non-the-less important chronic manifestations, are the thickened spermatic cords, fibrosed epididymis and tests which also occur with the infection.

The relationship between microfilaraemia rate and incidence of genital filariasis in the different endemic areas is clearly shown in Table 1. Scrotal elephantiasis and lymph scrotum occur more frequently in areas of high and hyperendemicity.

From Uganda, Burkitt (8) reported an extensive area where the incidence of hydrocoele approached 30 percent in adult males. No blood examinations were made, but as microfilariae of *W. bancrofti* were found in the fluid of 17 of 130 cases it is almost certain they were of filarial origin.

In Egypt, a village near Cairo, where the microfilaraemia rate in males was nearly 30 percent, was found to have a hydrocoele rate of 26 percent (28).

These rates are very much higher than those reported from West Africa, where McGregor and Smith (36), in the Gambia, reported «little hydrocoele» in an area where 40-60 percent of the adult population had microfilariae in their night blood. McFadzean (34), also from the Gambia, considered the incidence of hydrocoele low even with microfilaraemia rates of 19-38 percent, though in neither of these surveys were the genitalia of the male population examined. In the Cameroons, however, he considered hydrocoele to be more common. In Liberia, elephantiasis of the scrotum was the most frequently seen elephantoid condition (11).

Elephantiasis of the limbs is often considered to be synonymous with Bancroftian filariasis, and generally speaking is afforded much more attention than genital filariasis. Nowhere in East Africa, however, does the incidence of elephantiasis approach that of genital disease. The methods of survey used in Tanganyika prevented an accurate determination of the incidence of the condition being made, but in 3 villages on Ukara Island, where every person was examined, the incidence amongst adults was 2, 5 and 7 percent where the microfilaraemia rates were 20, 24, and 31 percent. Although no accurate figures for the incidence of elephantiasis in other parts of the territory are available, it is thought that the Ukara incidence is very much higher than the incidence elsewhere.

In Nyasaland rates of between 1 and 3 percent were noted by Oram (41). To the west of Lake Victoria, Stanley (50) noted the people of the area to be «happy, but suffering from elephantiasis». These cases have since been shown to be non-Bancroftian in origin (27). In other areas also, particularly in the highlands of Tanganyika and in Kenya (9), persons with elephantiasis are comparatively common, though Bancroftian infection is not found.

Elephantiasis rates of less than 1 percent were reported from the Gambia (34), but only 40-60 miles away in the French Cameroons McFadzean found 5.6 percent of the population with elephantiasis, although the microfilaraemia rate was only a little higher than that in the Gambia.

A 4 percent rate was reported by Fain (14) from Banningville Territory, in the Congo, but in Tangier (44), though filariasis is rare, elephantiasis and chyluria are not. In East Africa localised elephantiasis (52), abdominal abscess (13), lymphuria (25), chyluria (18) and haematuria (3) are also occasionally seen.

OBSERVATIONS ON IMMUNITY

The fact that infection rates of 100 percent in adults rarely, if ever, occur, even though the population may be exposed to several hundred infective bites every year (48), suggests that some people may have some degree of immunity. In the most heavily infected areas of Tanganyika, however, rates of over 60 percent in adults were found

on a single blood examination made several hours before the peak of the microfilarial tide. In these areas it is probable that nearly 100 per cent of people are infected and that any immunity had been overcome.

The higher incidence of infection in females has been referred to above. Franks (15) reported that persons of group A blood showed a higher incidence of infection with *W. bancrofti* than persons with other blood groups. A similar finding was reported with those infected with *Acanthocheilonema perstans* in Tanganyika (24). It has been reported that in one area where *Wuchereria malayi* and *W. bancrofti* co-exist a greater number of mixed infections than would be expected were found (6) — likewise, in an area of East Africa where *W. bancrofti* and *A. perstans* co-exist, in spite of different vectors, a higher incidence of mixed infections than would be expected by chance were found (23). These findings support the contention that certain people are more susceptible to filarial infections than others — or that some people have a degree of immunity to the infection.

ANIMAL RESERVOIRS OF INFECTION

Following the discovery of an animal reservoir of *L. loa* by Gordon (16) and the finding of *Wuchereria* sp. worms indistinguishable from *W. malayi* in dogs and cats (12), a renewed search has been made in East Africa for an animal reservoir of *W. bancrofti*. Dog and cat blood slides from many parts of Tanganyika have been examined, but only *Dirofilaria immitis* have been found. The blood of many large and small animals have also been examined, but no microfilariae resembling those of *W. bancrofti* have been seen. In Kenya, Heisch and Nelson (39) have found a «malayi» type microfilariae in domestic dogs, cats and genet cats, but since no human *W. malayi* infections have been reported, it seems most unlikely that their finding indicates an animal reservoir.

TRANSMISSION

A. gambiae (1, 17, 20, 28, 32, 37, 48, 53), *A. funestus* (17, 19, 20, 28, 32, 48, 53), *A. gambiae melas* (7, 37), *C. fatigans* (19, 46, 47)

and *Aedes aegypti* (20) have all been incriminated as vectors of Bancroftian filariasis in Africa. The efficiency of *A. aegypti* seems to vary from place to place and in experimental work with a Tanganyika strain it appears to be a poor vector.

It is difficult to compare the infection rates of one vector with another and different reports of the same vector owing to variations in the method of reporting the incidence of infection in the mosquitoes, but *A. gambiae*, *A. funestus* and *C. fatigans* all appear to be good vectors. In experimental work in the laboratory, however, when the smaller weight of the blood meal of *A. gambiae* is taken into consideration, this species appears to be a more efficient vector than *C. fatigans*. Recognised vectors of Bancroftian infection in different parts of the world have been shown to be capable of developing infective larvae when fed on blood containing very low levels of microfilariae (4, 45, 54) and the common African vectors are no exception to this. This finding is of importance when planning eradication schemes.

Low density infections are of further importance since neither the microfilariae imbibed by mosquitoes, nor the infective larvae developing from them will be in sufficient numbers to cause the death of the mosquito.

CONTROL

Vector control with insecticides over large tracts of Africa may be impracticable though the results of the Taveta — Para Malaria Control Scheme in Tanganyika will be awaited with interest. Control of mosquitoes in urban areas may, however, be possible.

Preliminary results of an attempt to control the microfilariae and probably the adult worms of *W. bancrofti* with diethylcarbamazine in Tahiti are encouraging (29), and preliminary results from a smaller pilot scheme in Tanganyika (26) likewise suggest that control in this manner may be possible. 100 mg of drug given monthly and 200 mg given every second month reduced the total number of microfilariae in infected persons by 89 and 93 percent respectively and 67 and 60 percent of persons showing microfilariae before treatment were negative at the end of 1 year's treatment. With 200 mg of drug monthly there was an overall reduction of 95 percent in the number of microfilariae present and 79 percent of infected persons became

negative. It was found that people having low microfilarial counts responded better to treatment than those with higher counts. The report from Gambia by McGregor and Gilles (35) that the incidence of negative bloods increases with time following a course of diethyl-carbamazine may indicate that it is unnecessary to treat carriers until the bloods are clear of microfilariae.

Even if follow up results in this small scheme in Tanganyika indicate that control is practicable, it has become apparent that, unless complete cooperation is given by the people, any extensive scheme involving distribution of drug monthly on a large scale in areas where compact villages do not exist is inevitably going to be time consuming, laborious and full of difficulties. Obviously, a combined attack on worms and mosquitoes is the best policy, but the additional expense of attacking both may be prohibitive in backward countries.

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GENERAL DISCUSSION

DR. JUSTIN ANDREWS (U.S.A.) — I understood Dr. Kessel to state that the present objective of antifilaria mosquito reduction was to pursue this to the level below which clinical manifestations of filariasis do not appear. (This was verified by Dr. Kessel.)

My questions then, compounded of ignorance and curiosity on my part, may be stated as follows: What is this critical level of transmission? In what terms is it defined? And what is the nature of the experimental or field observations on which it is based? — Or is this to be determined in the future by purely empirical means?

DR. B. O. L. DUKE (U.K.) — In many areas of Africa control of loiasis by the use of diethylcarbamazine is not possible. In such places the use of insecticides against *Chrysops* holds some promise of success.

The use of insecticides should be directed against the larvae, which live in mud, for these have a long life history extending over a period of at least one year.

Preliminary experiments have shown that the larvae are susceptible to insecticides and the method will be tried out first in the Cameroons rain forest where breeding gites through scattered are not in total area. Should insecticides succeed in this environment, it may be possible to adapt the method for use in the swamp forest type of breeding sites, common in Southern Nigeria.

DR. IAN A. MCGREGOR (U.K.) — For sometime my colleague, Dr. H. M. Gilles, and I have been observing in the Gambia, British West Africa, the effect of diethylcarbamazine on two small groups of Africans infected with Brancroftian filariasis. One group (50 individuals) live in a village which over the entire period of the obser-

vations has been sprayed annually with the residual insecticide dielchin. Further, this group represents almost all the infected persons resident in the village. The second group (36 individuals) live in a village untreated with residual insecticide and, at the time of treatment, was thought to represent the minority of infected residents.

Diethylcarbamazine at a total dosage of 12.5 mg base per kilogram of body weight was administered to both groups in 5 equal daily doses. The incidence of side effects at this treatment level proved almost negligible — this being in contrast to our experience in an earlier investigation in the same area, when diethylcarbamazine at a total dosage of 25 mg base per kilogram of body weight was given (McGregor, Hawking and Smith, *Brit. Med. J.*, ii, 908, 1952).

Following treatment, members of the group resident in the sprayed village were examined at 2, 9, 16, 28 and 43 months, when the percentage of individuals still exhibiting microfilaria in 50 wight blood samples was found respectively to be 74, 40, 27, 10 and 11. Members of the group resident in the unsprayed village were examined at 7, 19 and 33 months after treatment, when the respective infection rates were found to be 41, 30 and 36.

It is interesting to note that, whereas the response to treatment is similar in both groups over the first 2 years, the incidence of infection in the group resident in the unsprayed village begins to rise in the third year, while in the group resident in the village treated with residual insecticide it continues to fall, ultimately reaching a low level.

DR. W. KERSHAW (U.K.) — For the last 4 years an experiment has been in progress in the Cameroons and in the Niger delta to explore the possibilities of control of loiasis using only hetrazan.

During this time, the economic effect of the parasite (as estimated by the number of man-days work in this estate) has been reduced. Loiasis was the third most important cause of loss of time at the beginning of the experiment — it is now the seventh.

It is difficult, at present, to give the scientific results in detail, since many of the factors involved in interpreting the entomological data are obscure and since the natural fluctuations in the intensity

of transmission of infection are of the same order as the fall on the use of hetrazan. Cohorts of flies appear in batches and the transmission by young flies is much more efficient than that by older flies, since the older flies not only may not live long enough to transmit, but do not support the development of the parasite.

When such factors are better defined, the results of this experiment can be better interpreted.

Monday, September 8th — 3:00 to 4:30 p.m. Room D

The meeting was held under the chairmanship of Dr. Henri Galliard, in the absence of the appointed Chairman, Dr. E. H. Cluver. The Rapporteur was Dr. William Kershaw and the Secretary was Dr. Mário M. A. Abreu.

The following papers were presented and discussed.

A NEW GENUS, *BRUGIA*, FOR *WUCHERERIA* SPP.
OF THE MALAYI GROUP

J. J. C. BUCKLEY

When Brug in 1927 published his description of a new microfilaria, *Filaria malayi*, which he had discovered in the blood of man in Indonesia, there was considerable speculation as to what the parent worms would prove to be like. The microfilaria, which must have been frequently passed over formerly as that of *W. bancrofti*, was clearly very different in morphology from the latter species; so different indeed that it was reasonable to expect that this new form was not only a different species but that it belonged to a different genus.

It was with some surprise, therefore, that in the account of the discovery of adult worms of *Mf. malayi* in India by Rao and Mapleston (1940) one read the statement: «This worm is practically identical with *W. bancrofti* in nearly all its characters. The females are quite indistinguishable.» Their description of the specimens was brief and their illustrations of the male tail were not up to the standard which such an important discovery clearly necessitated. Differences were observed, however, in the spicules and in the anal papillae of the male worms, and in view of these points, together with the distinctive microfilaria and the different intermediate host (*Mansonioides annulifera*), the authors rather hesitantly named the

worms *Wuchereria malayi* in accordance with the name previously given to the microfilaria.

In 1941 Bonne *et al.* in Indonesia recovered adult worms from a human host; they described and identified them as *W. malayi*. Their line drawing of the important tail region of the male, though supplemented by a useful photograph, was inadequate, like those of Rao and Maplestone, and did not throw any new light on the true relationship between *W. malayi* and *W. bancrofti*.

In the meantime, Poynton & Hodgkin (1939) had discovered in the blood of Malayan monkeys (*Macaca irus*) microfilariae that were apparently indistinguishable from those of *W. malayi* in man; an important find which offered hopes of recovering and examining the adult worms from the animal hosts. (Re-examination of the specimens from human hosts in India and Indonesia has become impossible owing to their loss during the war.) Such hopes remained unfulfilled and, although the geographical range of *Mf. malayi* in man came to include many parts of the Far East, no adult worms were reported during the period 1941-1954.

In 1955, Edeson, Wharton and Buckley reported the finding in Pahang, Malaya, of microfilariae resembling those of *W. malayi* in the blood of dogs, cats and in 3 different kinds of primates, and that adult worms had been recovered from the lymphatics of some of these animals. Adults of 2 different species were described by Buckley & Edeson in 1956. One of these, named by these authors *W. pahangi*, was a natural infection in cats, dogs and the slow loris (*Nycticebus coucang*). It was subsequently reported by Edeson & Wharton (1958) as a natural infection also in the Musang (*Viverra zangalunga*), the tiger-cat (*Felis planiceps*) and the tiger (*Panthera tigris*). The other species, of which adults were recovered from *Macaca irus* and domestic cat as natural infections, were provisionally identified as *W. malayi* (Brug, 1927). Adults were also recovered from *Nycticebus coucang* and domestic cats which had been infected experimentally by inoculating them with infective larvae from mosquitos that had fed on infected human beings. This notable experimental transmission work, carried out by Edeson & Wharton (1957, 1958) in Malaya, represented the first successful transmission of a filarioid nematode from man to animals; and apart from other important consequences relating to the possible existence of reservoir hosts, it

has made numerous specimens available for study that are from a human source of infection.

In 1957, Nelson and Heisch discovered that dogs and cats on Pate Island, off the Kenya coast, harboured a *malayi* type of microfilaria in their blood—a new record from Africa. The adult worms were recovered from the lymphatics of these animals and proved to be a new species which was named *W. patei* by Buckley, Nelson and Heisch, 1958.

The adults of these 3 species, 2 from Malaya and 1 from Africa, as well as their microfilariae, have morphological features which unite them as a group and also serve to differentiate them from *W. bancrofti*. It is proposed, therefore, to remove them from the genus *Wuchereria* and to place them in a new genus, to be named *Brugia* in honour of the discoverer of *Microfilaria malayi*. The morphological features that characterize the new genus and differentiate it from *Wuchereria* are depicted in the diagram, and are summarized as follows:

1. The microfilariae of *Brugia malayi*, *B. pahangi* and *B. patei* are so similar that it is extremely difficult to differentiate them, though that of *B. patei* is distinctive in having a relatively short cephalic space. All 3 are easily differentiated from the microfilaria of *W. bancrofti*.

2. The left spicule in the 3 species of *Brugia* is characterized by a rather complex centre section which in *Wuchereria* is a simple structure.

3. The anal papillae in the male tail of the 3 species of *Brugia* are relatively few in number. Typically there is a total of 11 comprising 4 ventro-lateral papillae on each side, 2 papillae immediately post-anal and 1 large pre-anal papilla. Atypically there may be 5 or 3 ventro-lateral papillae. The anal papillae in *W. bancrofti* number about 24, and comprise 9 to 12 ventro-laterals on each side, 2 papillae immediately post-anal and 1 large pre-anal papilla. The term «anal papillae» does not take into account the intermediate papillae situated at a variable distance between the anus and the tip of the tail. In *Brugia* there are usually 2 of these and in *Wuchereria* there are about 4.

4. The species of *Brugia* are relatively small in size. The females attain 50-60 mm in length and a maximum width of 190 μ ; the males

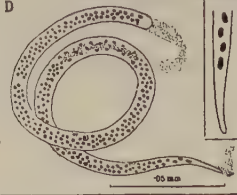
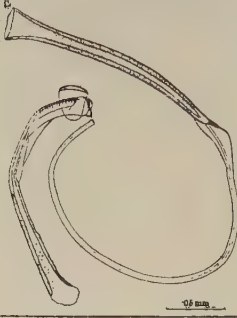
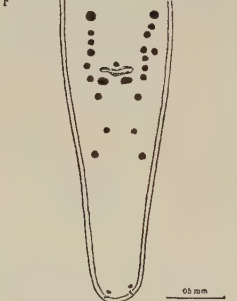
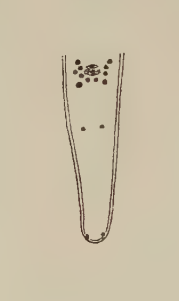
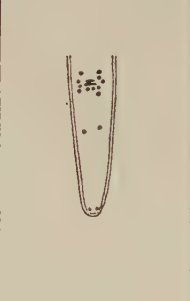
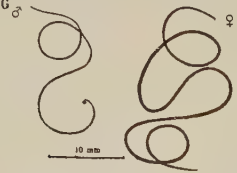
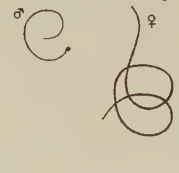
WUCHERERIA SILVA ARAUJO, 1877		BRUGIA GEN. NOV.	
<i>W. BANCROFTI</i> [COBBOLD, 1877]		<i>B. MALAYI</i> n. comb. [BRUG, 1927]	<i>B. PAHANGI</i> n. comb. [BUCKLEY & EDESON, 1950]
A MAN		MAN, CAT, MONKEYS, *LORIS *MUSANG	CAT, DOG, TIGER, LORIS, MUSANG, WILD CAT,
B CULEX spp. AEDES spp. ANOPHELES spp.		MANSONIODES spp. ANOPHELES spp.	MANSONIODES spp. ANOPHELES spp. ARABIDOTES sp.
C EQUATORIAL BELT		INDONESIA, MALAYA, INDIA, CEYLON, CHINA	MALAYA AFRICA
D 			
E 			
F 			
G 			

Fig. 1 — Illustration of the main differences between *Wuchereria* and *Brugia* gen. nov.: A, definitive hosts; B, intermediate hosts; C, geographical distribution; D, microfilariae, drawn to the same scale and (inset, magnified) tail nuclei of microfilariae; E, spicules, lateral view, drawn to the same scale; F, male tails, ventral view, drawn to the same scale; G, adult worms, actual size

* Experimentally infected only.

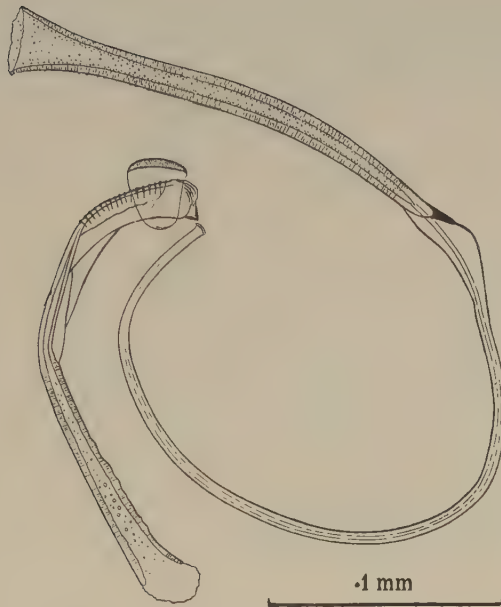


Fig. 2 — Spicules and accessory piece of *Wuchereria bancrofti*

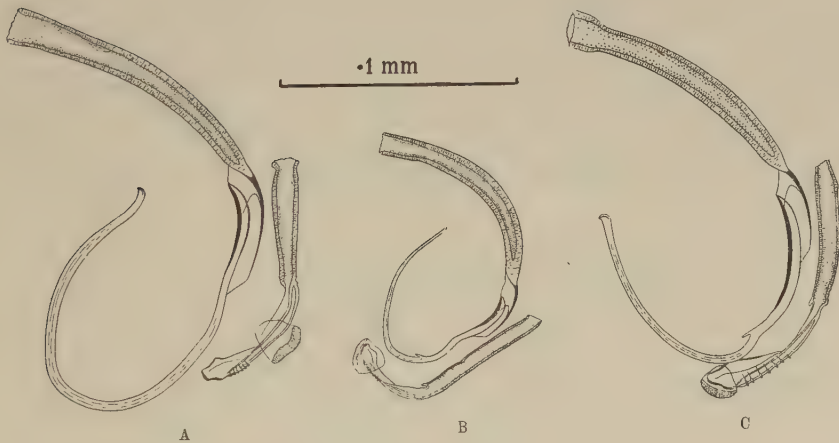


Fig. 3 — Spicules and accessory piece of: A, *Brugia malayi*; B, *Brugia pahangi*; C, *Brugia patei*. (The delicate membrane usually attached to the distal part of the left spicule in all 3 species, including that of *W. bancrofti*, has been omitted from these figures)

attain about 25 mm in length and are less than 100 μ in maximum width. *Wuchereria bancrofti* attains 100 mm in length and 300 μ in maximum width in the female; the males are nearly 40 mm in length by about 120 μ in maximum width.

DIAGNOSIS

Dipetalonematinae Wehr, 1935: Relatively small filarioid nematodes having affinities with *Wuchereria bancrofti*. Cuticle smooth except on the ventral surface of the male tail, which is transversely striated, and on the posterior part of the female, which may bear minute cuticular tubercles. Body tapers anteriorly and terminates in a well-defined head bulb. Cephalic papillae in two rows, the anterior row comprising a subventral and a subdorsal pair, between which is a pair of lateral amphids; the posterior row comprises one subventral pair and one subdorsal pair. Mouth opening simple, circular. Oesophagus indistinctly divided into two parts; anterior extremity cuticularized at its junction with the mouth opening.

Female: Vulva in the oesophageal region, joined to the vagina by a conspicuous pear-shaped ovejector; vagina long and usually bent on itself several times; uteri uncoiled, running side by side from the vagina to the ovarian tubules, which they join in the posterior part of the body; microfilariae sheathed, with characteristic terminal tail nuclei; tail rounded, with a pair of subterminal phasmids and single left lateral phasmid situated between the anus and the tail extremity.

Male: Adanal papillae typically comprising 4 pairs of lateroventrals; a post-anal pair immediately behind the anus and a single large pre-anal papilla immediately in front of the anus; intermediate papillae, usually 2 in number, at a variable distance between the adanal group and the tail tip; tail tip with 2 or more papillae; phasmids also present. Spicules unequal and dissimilar. Left spicule in 3 parts, a tubular proximal part, coarsely stippled; a complex central unstippled part; a distal lash-like part, which may or may not have a spatulate termination; attached to the distal part is a delicate membrane. Right spicule with finely stippled tubular proximal part and complex distal

part, semitubular, through which the left spicule passes; with transverse dorsal corrugations at its extremity. Finely stippled crescentic accessory piece present.

Parasitic in lymphatic system of primates, carnivores and insectivores; microfilariae in circulatory system.

Genotype: *Brugia malayi* (Brug, 1927).

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CHEMOTHERAPY OF FILARIASIS: PERIODICITY OF MICROFILARIAE ¹

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CHEMOTHERAPY

Since 1953 no major developments have been made in the chemotherapy of filariasis. The subject has been reviewed in 1955 (Hawking, 1955). Accordingly, only a brief summary of the present position will be given here. At the present time, there are only two compounds of practical value in the treatment of filariasis, *viz.* diethyl carbamazine and suramin.

Diethyl carbamazine, 1-diethyl carbamyl-4-methyl piperazine, is also known as hetrazan, banocide, notezine, caricide, carbilazine, 3, 799 R.P. Its main action is upon the microfilariae, which are destroyed by it *in vivo* but not *in vitro*; its effect upon the adult worm varies according to the species. It appears to kill (or permanently sterilise) the adult worms of *Wuchereria bancrofti*, *W. malayi*, and *Loa loa*, but it has little or no effect upon the adults of *Onchocerca*, *Mansonella ozzardi*, *Dirofilaria* or *Litomosoides*, and only a slight effect on *Dipetalonema perstans*.

When diethyl carbamazine is given by mouth, it is rapidly absorbed and much of it (70 percent) is excreted in the urine in 24 hours. The metabolism of the compound has been investigated by Bangham (1955) using drug labelled with C¹⁴. Partial breakdown of the side chains occurs quickly, but the piperazine ring remains intact.

¹ This paper is a review of present knowledge and views about chemotherapy and about periodicity with especial reference to developments since the last International Congress in 1953.

The therapeutic activity seems to be due to the intact molecule and not to any of its degradation products. The compound is not concentrated either in the adult worms or in the microfilariae to any significant extent.

Diethyl carbamazine may cause minor toxic effects, *viz.* direct effects, observed in uninfected subjects (such as anorexia, nausea, vomiting, headache and sleepiness), and indirect allergic reactions (such as inflammation of the deeper tissues round adult worms, pyrexia, and general malaise). These allergic reactions are particularly marked in patients infected with *Onchocerca*, less so with *W. malayi*, and *L. loa* and less so again with *W. bancrofti*. They are probably an allergic reactions to the liberation of antigens from the microfilariae which have been destroyed, and they are said to be diminished by cortisone or prednisone (Markell & Turner, 1957). They may prove a handicap in the conduct of mass campaigns. As regards major toxicity, diethyl carbamazine has been remarkably free, and no deaths due to it have ever been reported.

In practice, diethyl carbamazine has been employed in two ways:

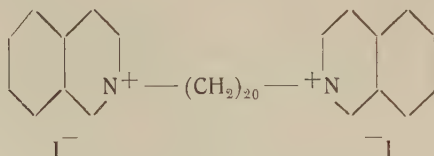
- a) For individual patients infected with *Loa*, *W. bancrofti*, or *W. malayi*, in all of which it usually produces a permanent cure. It has also been widely used for patients infected with *Onchocerca*; in these it is valuable in destroying microfilariae in the skin and in preventing damage to the eyes, but the microfilariae usually return after a variable interval.
- b) In mass campaigns to control or eradicate *W. bancrofti* or *W. malayi*. For this purpose it has been very valuable; but detailed discussion is left to another session of the Congress.

Suramin is also known as Bayer 205, antrypol, naphuride sodium, moranyl, germanin, belganin, etc. It is effective against *Onchocerca volvulus*, but it has little action against *Wuchereria*, *Loa*, *D. perstans*, *D. streptocerca*, *Dirofilaria* or *Litomosoides*. The recommended dose is 1 g intravenous injection weekly for 5 weeks (or, in resistant cases, 10 weeks). Many of the toxic effects reported in the early series were due to doses higher than this; even in smaller doses it is a potentially toxic compound which should be used with discretion and restraint. It kills the adult worms, after which the microfilariae gradually

die out. Its chief practical use is for the treatment of individual patients with onchocerciasis, preferably in association with diethyl carbamazine. As to whether it is suitable or safe for mass campaigns to eradicate or control onchocerciasis, conflicting reports have been given. There is great need for a safe, effective, and cheap drug for this purpose.

Other compounds active in the laboratory. — Various other compounds have shown antifilarial activity in the laboratory when tested upon *Litomosoides*, but they have failed to show practical value in man. Pentavalent antimonial, e.g. neostibosan, will usually kill the adult worms of *W. bancrofti*, but the dosage must be high approaching the toxic level. Trivalent arsenicals, especially arsenamide (thiarscetamide), are also effective in killing the adult worms of *W. bancrofti*. But in some patients arsenamide has a toxic action upon the liver and one death due to acute liver necrosis has been reported, so that this treatment is too dangerous for a non-fatal infection such as filariasis. Cyanine compounds and methylene violet have proved active against *Litomosoides*, but they have no practical effect upon human filariae.

Recently, in collaboration with Dr. H. O. J. Collier, Dr. E. P. Taylor and Dr. R. J. Terry, we have found that symmetrical bis-isoquinolinium compounds of the type BIQ 20 iodide



have considerable activity against *Litomosoides* in cotton rats. The minimal effective dose is 0.15 mg/kg given i.p. daily for 5 doses; while the 50 percent lethal single dose for mice is 2.8 mg/kg. The main action of these compounds is exerted upon the adult worms, rather than upon the microfilariae. They have not yet been tested upon the human filariae, but preliminary studies have been made upon *Dirofilaria* of dogs by Prof. P. C. Beaver of New Orleans.

Mode of action of antifilarial drugs. — In my laboratory, we have devoted much attention to the mechanisms by which antifilarial drugs

produce their effects. Diethyl carbamazine (which works chiefly against the microfilariae) is remarkable in that it sensitises the microfilariae in some way (rather like an opsonin), so that they are seized and destroyed by the large phagocytes of the reticulo-endothelial system (Hawking, Sewell & Thurston, 1950). The other antifilarial drugs attack the adult worms. Work by Miss A. E. R. Taylor has shown that the first parts of the worm to be affected are the oocytes and developing embryos in the uterus. (The exact details differ with the various drugs.) These may be disorganised and destroyed, while the worm as a whole remains actively motile showing that the neuromuscular mechanisms are still intact. The oocytes and developing embryos possess the most active metabolism (especially anabolism) of all the tissues of the worm, and this may explain their especial susceptibility to antifilarial drugs.

PERIODICITY OF MICROFILARIAE

Causation of periodicity.—The other subject to which it is desired to draw the attention of the Congress is the periodicity of microfilariae. We have studied this phenomenon for many years, especially by (a) counting microfilariae in sections of the different organs so as to map out their distribution at different times and (b) observing the effect of physiological stimuli such as exercise, breathing oxygen, etc., upon the number of microfilariae in the blood by day and by night (Hawking & Thurston, 1951; Hawking, 1956; McFadzean & Hawking, 1956). This work has shown that periodicity depends upon the microfilariae, and it occurs even when the adult worms are absent (Hawking, 1953). When microfilariae leave the peripheral circulation (e.g., during the day), they accumulate in the capillaries and small vessels of the lungs; and periodicity depends upon a migration backwards and forwards between the lungs and the periphery. There is a passive phase in which the microfilariae are fairly evenly distributed throughout all the blood, and hence they appear numerous in the peripheral circulation; and there is an active phase in which the microfilariae are concentrated in the lungs. The purpose of the passive phase is to give an opportunity for the microfilariae to be sucked up by an insect vector and it is timed accordingly; (with microfilariae

transmitted by nocturnal mosquitoes, it occurs at night; with *Loa*, which is transmitted by diurnal *Chrysops*, it occurs by day). The purpose of the active phase (of concentration in the lungs) is less clear, but we must assume that the lungs are the optimum site in the body for the survival of the microfilariae. The distribution of microfilariae between lungs and periphery probably depends upon a dynamic equilibrium rather than on a static one; the total number in the lungs may remain constant over appreciable periods, but the individuals probably keep changing. There are always many microfilariae in the lungs, even when the number in the peripheral blood is maximal. The concentration of microfilariae in the lungs does not depend upon any opening or shutting of capillaries by the host (since *L. loa* and *W. bancrofti* behave in opposite manners in the same host), but it must depend upon the microfilariae themselves giving an active response to some stimulus or stimuli provided by the 24-hour rhythm of the host. The nature of this active response, whereby the microfilaria holds itself in the capillary of the lung, is mysterious and requires further study. Recent experiments by Mr. S. R. Smithers have failed to show any relation to electrostatic charge (periodic microfilariae like *Dirofilaria immitis* showed no electrostatic charge, although non-periodic ones like *Litomosoides carinii* were negatively charged).

As regards the stimuli which are effective in lowering or raising the number of microfilariae in the peripheral blood (i.e., increasing or decreasing the number in the lungs), we have studied 10 different species of microfilariae with many different stimuli (Table). Several stimuli have been found which produce marked changes in the microfilaria count. Thus, if a man containing African *Mf. bancrofti* is given oxygen (40-100 percent) to breath at night, the number of microfilariae in the peripheral blood diminishes to about 20 percent of its initial value in about 1-2 minutes, and rapidly returns to its previous level when the oxygen is discontinued (McFadzean and Hawking, 1956). With the Pacific variety of *W. bancrofti* (which is non-periodic) breathing oxygen does not cause a fall in the microfilaria count, but rather a rise (Edeson, Hawking & Symes, 1957). It is difficult however to explain the periodicity of *W. bancrofti* on these lines, since the experimental change in the oxygen pressure must be at least 70 mm Hg., while the natural change in the oxygen pressure

The reaction of microfilariae of various species to different stimuli

Stimulus	Day-time								Night-time								
	African <i>W. bancrofti</i>	Pacific <i>W. bancrofti</i>	<i>W. malayi</i>	<i>L. loa</i>	<i>A. persians</i>	<i>D. acbiops</i>	<i>D. immitis</i>	<i>D. repens</i>	<i>Dip. blanci</i>	<i>L. carinii</i>	African <i>W. bancrofti</i>	<i>W. malayi</i>	<i>L. loa</i>	<i>A. persians</i>	<i>D. acbiops</i>	<i>D. immitis</i>	<i>D. repens</i>
Increased oxygen.	0	+	--	0	0	+	+	+	+	0	--	--	0	0	+	+	+
Hypoxia	0	+		0	0	+	+	+	?	0	0		0	0	+	+	+
Increased carbon dioxide	0	0	0	0	0	+	0	0	0	0	+	-	0	0	0	0	-
Hyperventilation	0	0	-	0	0	0	-	0			-	-	0	0	--	--	?
Exercise	0	--	-	-	0	0	0	+			-	-	0	0	-	+	?
Ammonium chloride infusion	0					+					0				-	+	
Sodium bicarbonate infusion	0				0	0		?			0				-	?	?
Anaesthetic	0			-		+	+	+	+		?				+	+	+

+ or ++ indicates that the microfilarial count in the peripheral blood increased slightly, or considerably, respectively.
 - or -- indicates that the microfilarial count in the peripheral blood decreased slightly, or considerably, respectively.
 0 indicates that the microfilarial count in the peripheral blood did not show any significant change.

in the alveolar air during the 24 hours is only about 5 mm Hg. With *Dirofilaria aethiops* of monkeys, *D. immitis* and *D. repens* of dogs, and *Dipetalonema blanci* of gerbils, oxygen causes a marked increase in the microfilaria count. It has no effect upon *L. loa*, upon *A. perstans* or upon *Litomosoides*. A sharp fall in the microfilarial count of *W. bancrofti* in man at night can also be produced by making the man take violent exercise, but this does not affect *L. loa*, or the *Dirofilariae* of dogs. Altogether it seems that microfilariae of different species respond differently to many stimuli; and it may well be that the stimuli responsible for their periodicity may also differ according to species.

Passage through capillaries. — The passage of microfilariae through the usual capillaries is mostly a passive affair. It has been observed microscopically *in vivo* by myself and Dr. J. A. McFadzean with *D. repens*, and more recently by my colleague Miss A. E. R. Taylor, who has studied *Litomosoides carinii* passing through the capillaries of the liver under an Ultrapak objective. The microfilariae are carried down the capillaries as rapidly as the red blood corpuscles. Exceptionally when the circulation is slowed, either by closure of a vessel or by antemortem changes, microfilariae (both *Dirofilaria* and *Litomosoides*) may progress slowly against the stream for a short way. More recently, however, we have had an opportunity of studying a very large microfilaria — about 600 μ long — which occurs in a Malayan monkey, *Macaca irus*. The number of microfilariae found in the peripheral blood by puncture of the skin in different parts of the body was highly irregular and it was not significantly increased by stimuli, e.g., oxygen, anoxia, anaesthetic, which we had found the small vessels of the skin (probably increased by stasis of the local circulation); in histological sections of the skin, the microfilariae could be found accumulated in arterioles but not in venules. In this instance, the great length of the microfilaria seems to cause some difficulty in its passage through the capillaries.

Turnover and destruction of microfilariae. — Finally consideration may be given to the turnover and ultimate fate of the microfilariae which are found in the blood stream. In the case of *Dirofilaria repens* effective with other species. The microfilariae seemed to congregate in

one adult female worm may produce 5,000 microfilariae per day (Webber & Hawking, 1955), and one adult *Litomosoides* produces 15,000 microfilariae per day (Hawking, 1954). The average life of a microfilaria of *W. bancrofti* is probably 6-9 months (based on results with neostibosan, Culbertson *et al.*, 1947), of *A. perstans* about 3 years (Gonnert, 1942) and of *Mansonella ozzardi* about 2 years (Mazzotti & Palomo, 1957). Microfilariae of *Dirofilaria repens* in dogs live from 2 months (Hawking, 1953) to more than 2 years (Fülleborn, 1929). Accordingly the large numbers often found in the blood are probably due to accumulation over a long period. Very few of these microfilariae ever reach an insect vector and fewer still pass on to complete development in a new host. All the rest must perish in the body of the original host, but evidence for their destruction is remarkably small. Apparently they are so well adapted to their environment, that the host tissues do not regard them as foreign bodies, and little or no immune reaction is directed against them. (*L. loa* is the least well adapted of the human filariae, and immunological reactions against it are more marked than against the others.)

During our extensive examination of histological sections, we have occasionally found small granulomatous foci in the lungs and other organs, where a few microfilariae seem to be undergoing dissolution; but these could account for only a small fraction of the microfilariae to be destroyed. It has recently been found by Dr. B. O. L. Duke (private communication) that in monkeys infected with varieties of *Loa* excision of the spleen results in a large and prolonged rise in the microfilarial count of the blood; presumably the number in the bloodstream had been kept down by continual destruction in the spleen. As noted above, *Loa* is the least well adapted of the well known filariae, and it is probable that this defence mechanism is less well developed with other filariae. In view of Duke's results we have examined the effect of splenectomy in dogs infected with *D. immitis* and *D. repens*. In half the dogs there was no change, in the microfilarial count; in the other half there was a gradual and moderate rise which subsided again after a few months. Apparently the spleen is less active in destroying microfilariae of *Dirofilaria* in our dogs than it is in Duke's monkeys with *Loa*. This question of the destruction of microfilariae deserves more consideration and study.

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STUDIES ON IMMUNITY TO THE FILARIAL WORM OF COTTON RATS

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Since very few investigators have reported on work on immunity to filariasis in animals in recent years, this report will necessarily deal mainly with the work of one laboratory. In this connection I wish to acknowledge the contributions to this report of my colleagues and students. Complete immunity to filarial worms has not been demonstrated in any laboratory animal, as Bertram (1953) has stated. It is assumed that he referred to acquired immunity developing in so-called normal hosts. McFadzean (1953), after reporting his experience and reviewing the literature, concluded that specific immune reactions to filarial worms have proved difficult to demonstrate and are slight in extent as compared with reactions to some other nematodes.

In our laboratory experimentation on a quantitative basis has been made possible by the introduction of a method for infecting cotton rats with *Litomosoides carinii* by dissecting infective larvae from the vector, the tropical rat mite, and administering them subcutaneously in counted numbers. One advantage of this method is that the percentage of larvae which develop can be determined. On the average about 55 percent develop in cotton rats which have not been previously exposed to infection (Scott and Macdonald, 1953). The variability between experiments is quite high, probably because of our inability to completely standardize the physical conditions which affect the viability of larvae during administration. Practically all of the larvae which complete the migration from the skin to the pleural cavity, or more rarely to the abdominal cavity, continue to grow to maturity. None of the immunizing procedures which are

described below have produced a significant change in the percentage development in cotton rats. A second advantage of the quantitative method is that, because of the simultaneous administration of a lot of larvae, the rate of growth and development of the worms can be assessed (Westbrook and Scott, 1955).

ACQUIRED IMMUNITY IN COTTON RATS

Worms of a second infection superimposed on a primary infection were retarded in growth and development as compared to worms of parallel primary infections (Macdonald and Scott, 1953). We have called this retardation of growth an expression of immunity, since it apparently is good evidence of a power to resist development of infection arising in response to previous infection. If the secondary or challenging infection is given at least 30 days after the primary or immunizing infection, then 38 days later the two groups of female worms from the two infections can be distinguished by the difference in length. At 24 days after the second infection the same type of differentiation can be made for both sexes. This latter time also corresponds with the occurrence of the final molt. The percentage of worms which have at least started the molt by this time provides a measure of morphological development. From measurements of length the rate of growth can be assessed.

Since the two infections can only be differentiated by size, it has not been possible to determine whether this early retardation of growth in immunized animals persists through the rest of the growth period and results in stunted mature worms, or whether they eventually reach the same size as worms in primary infections but do so more slowly. The experiments of Bertram (1953) indicate that they become permanently stunted. He found that the reproductive capacity and ultimate length of female worms were reduced in animals given multiple infections as compared with those in animals receiving single infections. He appears to explain his results in terms of what we are calling immunity, since he states that «inhibition of growth and production of the female worms [...] is a clear indication of some adverse factor associated with reinfection». On the other hand, he states elsewhere in the same paper that in singly infected rats «the

growth of females is retarded, possibly also their reproduction, by overcrowding, or some factor related to numbers». The evidence from our experiments indicates that the retardation of growth in the early stages, at least, is not the result of crowding. The retardation increases in proportion to the number of worms in the immunizing infections, but is not related to the size of the challenging infection. Moreover, in a large series of single infections there was no significant inverse correlation between the length of the worms and the number of infective larvae administered (Westbrook and Scott, 1955).

Another series of experiments showed that the immunity expressed as retarded growth acts on the worms primarily in the early stages of their development (Scott *et al.*, 1958b). Infective larvae were administered to previously uninfected cotton rats and allowed to develop for 7 days. The worms were then removed from half of the cotton rats and transferred surgically to cotton rats which already harbored infections. After another 7 days they were found to be just as far advanced as worms which had remained for the entire 24 days in the same cotton rats. On the other hand, when the worms spent the first 7 days in previously infected cotton rats and were then transferred to previously uninfected rats for 17 days, their growth was found to have been significantly retarded. The infective larvae are about 0.8 mm in length and grow to about 1.1 mm during the first 7 days in previously uninfected cotton rats. Since the deficiency in growth of worms transferred from immunized animals was about 2 mm, most of the retardation must have occurred during the 17 days after transfer to the non-immune cotton rats.

Since it was found that immunity acts primarily on the early developmental stages, an attempt was made to determine which stages stimulate the immunity (Scott *et al.*, 1958a; Scott and Macdonald, 1958). Worms were transferred after 7 to 28 days growth in one group of cotton rats to a second group of previously unexposed cotton rats. After another 30 days these latter animals were challenged in the usual way and the challenging worms measured after 24 more days. Only when the number of transferred worms was relatively large was there any retardation of growth of the challenging infection. In every case the retardation was greater among worms of parallel infections in cotton rats immunized by the subcutaneous or intraperitoneal introduction of infective larvae equal in number to the transferred worms.

It seems clear that the principal immunizing effect is produced by worms during their first 7 days of development.

In view of these findings, it was of interest to determine how long the effect of a single infection would persist. Cotton rats were given a single infection and protected against further infection for one or two years. Challenging infections given after these long periods still showed a significant retardation of growth even though the immunizing infection had in some cases been dead for a long time (Macdonald and Scott, 1958). The question must be left open as to whether the immunity was produced by the original infection during the first 7 days and persisted without reinforcement, or whether the presence of live mature worms or even dead worm tissue could have some reinforcing effect.

Another type of immune response is the appearance of oral precipitates on infective larvae of *L. carinii* placed in sera of infected cotton rats and the absence of such precipitates in sera of uninfected hosts (Scott, 1952). In sera of cotton rats infected with a small dose of larvae the frequency of occurrence and the amount of precipitate found are minimal as compared with the response obtained after either a large single dose or multiple small doses (Briggs, 1958). When cotton rats received, by surgical transfer on three separate occasions, worms which had developed 7 days in other cotton rats, their sera produced only traces of precipitate as compared with the abundant precipitates produced in sera of control cotton rats immunized with equal numbers of infective larvae administered in the same way and on the same schedule. Evidently the power to produce precipitates is stimulated in cotton rats principally by worms in the early stages of development.

NATURAL IMMUNITY IN WHITE RATS

The laboratory white rat has been shown by several observers to have a high degree of natural immunity to this parasite of the cotton rat. Olson (1957) has shown that at 1 month of age white rats are just as susceptible as cotton rats. Whereas the latter continue to be susceptible throughout life, the white rats show considerable resistance at 2 months of age, and soon thereafter develop a high degree

of resistance. In mature white rats less than 10 percent, and usually less than 1 percent, of the infective larvae introduced subcutaneously complete the migration to the pleural cavity. Those which do develop are greatly retarded in growth. For example, female worms 24 days after infection measured 4 mm in length in white rats and 11 mm in cotton rats (Briggs, 1958). By this time a layer of host cells has begun to accumulate on many of the living larvae in the white rats. Practically all of the worms die before reaching sexual maturity and are completely encapsulated by these host cells.

In striking contrast to this picture, when larvae were allowed to develop for 7 days in cotton rats and were then transferred surgically to white rats, they developed normally and produced microfilariae over a long period of time (Olson *et al.*, 1955). Evidently, natural immunity, like acquired immunity, acts primarily on the early stages of development.

The question was raised as to whether the *in vitro* precipitates associated with acquired immunity in cotton rats would also be associated with natural immunity in white rats. No precipitates appeared in any of a large number of sera from white rats which had never been exposed to infection. About 2 weeks after exposure to infection, however, precipitates did appear, just as they do in cotton rats. One striking fact is that the amount of precipitate was not related to the number of worms developing, but to the number of infective larvae administered. In one case precipitates formed in abundance even though no developing worms could be found in the pleural cavity (Briggs, 1958).

When cortisone was administered to white rats daily beginning 6 days before infection, about 40 percent of the infective larvae were found to be developing in the pleural cavity 24 days after infection. At most only one worm was found in parallel white rats given equivalent injections of saline. When measured, the worms from the white rats receiving cortisone were not quite as long as those from parallel cotton rats given saline, but were much longer than those from the white rats given saline. Moreover, there were only slight traces of precipitate formed in sera of the white rats given cortisone, whereas abundant precipitates formed in the sera of the other two groups (Briggs, 1958). Although these results show that cortisone

will suppress a large part of the effects of natural resistance, and other experiments show that it will suppress the encapsulation of developing worms (Olson, 1957), there was some residual resistance which was not suppressed by cortisone in the doses given.

DISCUSSION

Many species of helminths show a retarded rate of growth in immune animals. Of the hypotheses suggested to explain this retardation, most of the evidence has seemed to favor one based on the effect of the precipitates which have frequently been demonstrated *in vitro* and in some species also *in vivo*. The nutritional level may be depressed through loss of digestive enzymes, as suggested by Chandler (1939), since these enzymes appear to act as antigens which combine with antienzymes acting as antibodies to form the precipitate. Although *in vivo* precipitates have not been found in association with *L. carinii*, they may occur.

The power to form precipitates is induced in the host primarily by worms in their early stages of development, i.e., while they are undergoing migration through the tissues. They may at this time be secreting maximum quantities of enzymes to aid in migration, or the antigens may be absorbed more effectively because of the intimate contact with tissues. The lack of precipitates in sera of white rats treated with cortisone may result from an inhibition of antibody production, a well known effect of this hormone. The fact that cortisone treatment of these animals also increased the number which completed migration raises a more difficult question. It can hardly be assumed that the migrating worms are immobilized by precipitate formation in the naturally immune white rat, as is the case with other species of nematodes in animals with acquired immunity. No precipitates were found *in vitro* until 2 weeks after exposure to infection, whereas migration is usually completed in 6 days or less. Perhaps cortisone merely inhibits inflammatory reactions which interfere with migration, but why these reactions would occur in the abnormal host and not in the normal host is not clear.

The partial inhibition by cortisone of the growth retarding effect of natural immunity is still more difficult to explain. The

transfer experiments in white rats show that the growth retardation is induced in the first 7 days, too soon for the precipitate producing antibodies to be found. Moreover, the transfer experiments in cotton rats showed that the growth retarding effect in immune animals acted on the worms in the first 7 days, but the retardation actually occurred after the worms had been transferred to non-immune hosts. It does not appear likely that a deficiency of nutrition resulting from destruction of enzymes through precipitation with antibodies would be carried over by the worms into a non-immune host. Some hypothesis involving suppression of enzyme production would be more in line with the facts to be explained, but as yet there is no evidence to support such an assumption.

Obviously much more experimentation will be needed to supply sufficient evidence to support any hypothesis to explain the retarded growth resulting from both natural and acquired immunity. The hypothesis based on interference with nutrition by precipitation of digestive enzymes has seemed to be reasonable and supported by considerable evidence. In the case of *L. carinii*, however, the contradictory evidence indicates that either a modification of this hypothesis or an entirely new one will be needed.

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SEROLOGICAL INVESTIGATIONS WITH *LOA LOA* ANTIGENS

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For checking filarial antibodies in human sera the antigen of *Dirofilaria immitis* worms from the dog as introduced by Fairley (1931) has proved to be favourable in the routine complement-fixation-test. This antigen is an alcoholic extract from dried, powdered *Dirofilaria* worms. These filariae of animal origin are big enough and the easiest to be obtained for the said purpose. The serological reaction is a filarial group specific one.

It was of interest to try the antigenic efficiency of human filariae against sera of homologous and heterologous infections whether a species specificity could be proved. As far as I know, only van Hoof (1934) worked in the CFT with an antigen from human filariae. He tested an extract of *Onchocerca volvulus* against sera of *Volvulus* infections.

Some time ago in the Cameroons I was able to test in the CFT different kinds of antigens from *Loa loa* worms (monkey strain). Similar tests with *Loa loa* antigen (human strain) were carried out in Hamburg. As far as I am aware, antigens of *Loa loa* have never been tried before in the CFT against homologous and heterologous sera.

Dr. Kershaw, Liverpool, was so kind to supply me the *Loa loa* worms (human strain), and Dr. Duke, Kumba, in the Cameroons, helped me to obtain these worms from the human and the monkey strain.

Duke (1957) reported from the Cameroons about infections with *Loa loa* in monkeys. The adults and the microfilariae are not morpho-

logically distinct from the human strain, but the microfilariae show a nocturnal periodicity. It was of interest to find out whether both strains differed serologically in the CFT. After using D'Alessandro and Sofia's absorption technique with kaolin, sera of man infected with *Loa loa* (human strain) and of monkeys experimentally infected with *Loa loa* (monkey strain) cross-tested against *Loa loa* (human strain) antigen and *Loa loa* (monkey strain) antigen turned out to be negative for filarial antibodies. Both an alcoholic *Loa loa* antigen (monkey strain) and an alcoholic *Dirofilaria immitis* antigen gave negative results in the final CFT. Absorption tests with *Dirofilaria immitis* antigen against *Loa loa* sera (human strain) and *Loa loa* sera (monkey strain) proved to give only a reduced titre in the CFT against *Dirofilaria immitis* and *Loa loa* antigens. According to these results, there was no serological difference between *Loa loa* (human strain) and *Loa loa* (monkey strain).

If the saline solution, in which living *Loa loa* (human strain) had been deep frozen, was used as an antigen, only *Loa loa* sera reacted positive, whilst sera from persons with other filarial infections proved to be negative. *Loa loa* sera showed a titre up to 1:32, whereas sera of infections with *Wuchereria malayi* and *Dracunculus medinensis* reacted only up to 1:2. Saline solution of frozen *Loa loa* worms (monkey strain) turned out to be positive with sera from infections with human and monkey *Loa loa* strains. From these results it can be taken that a filarial species specific serological diagnosis seems to be possible, using the antigenic power of the metabolic substances, which enter into the saline solution from living frozen filariae.

An alcoholic *Dirofilaria immitis* antigen from powdered worms has been compared with a *Loa loa* antigen (monkey strain) prepared in the same manner against sera from people from a village in Nigeria where no other human filarial infections are known but *Loa loa*. Persons with *Loa loa* microfilariae were positive with the *Dirofilaria immitis* antigen in 78.6 percent and with the *Loa loa* antigen in 86 percent. Persons without *Loa loa* microfilariae were positive with the *Dirofilaria immitis* antigen in 50 percent, and with the *Loa loa* antigen in 61 percent. Even if there is a higher percentage of positive reactions with the *Loa loa* antigen, the work of Ridley (1956) has to be mentioned who reported 85 percent positive CFT results in loiasis,

using *Dirofilaria immitis* antigen. Sera from Europeans infected in the Far East with *Wuchereria bancrofti* or *W. malayi* showed the same results with the *Dirofilaria immitis* and the *Loa loa* antigens. These results demonstrate that an alcoholic extract of *Loa loa* worms (monkey strain) seems to be somewhat more favourable against sera of homologous infections than a *Dirofilaria immitis* antigen. The mentioned *Loa loa* antigen did not work species specific, but filarial group specific just the same as the *Dirofilaria immitis* antigen.

THE TAXONOMIC SEPARATION OF MICROFILARIAL SPECIES

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At present the differentiation of the larval forms of nematodes, termed microfilariae, into taxonomic species rests upon data presented by Earnest C. Faust, in 1949, in his book *Human Helminthology*. According to Faust, measurements, if correctly carried out, may be used to separate species of human microfilariae. He made a series of measurements from the anterior end of a microfilaria to the beginning of each of the four major internal landmarks: namely, the nerve ring, excretory pore, first genital cell and anal pore. These distances he expressed in terms of their relative percentage of the total length from the anterior end of the worm, and said: «The relative distances of these landmarks from one another along the longitudinal axis, together with the size and relation of length to breadth, are utilized in specific diagnosis [...] since they are constant in the same species.»

About 9 years ago the speaker first became interested in microfilariae, while a collaborator with the U.S. Fish and Wildlife Service of the Department of the Interior, and working on the blood parasites of ducks, geese, quail, and wild doves and pigeons. While becoming familiar with the literature on the microfilariae of birds, it became evident that the entire subject was in a state of considerable confusion. Few complete life histories of avian nematodes have been worked out, and not many of the adult worms have even as yet been found in hosts from which the larval microfilarial forms have been reported.

With almost no exceptions, most writers recorded only the presence or absence of these larvae in a bird's blood, with perhaps their

overall length and width measurements, sheath, and the shape of their ends, to separate one from another. The literature became sprinkled with references to Dr. A's microfilariae, named «Form A», «Form B» and «Form C», as well as Dr. B's «Form A», «Form B» and «Form C», Dr. C's «Form A», «Form B» and «Form C», and so on. It was then, and still is, impossible to know whether all of these mentioned microfilariae were in fact different species, or whether any were the same, and, if so, which ones.

Certainly the separation of microfilarial species upon the basis of their differing in overall length and width has never been valid, since it is known that their size varies considerably during their stay in the blood stream. It has all too often become the habit, presumably so that some little order could be made out of the existing chaos, simply to assume that each microfilaria found in a new avian host was separate and different from any formerly reported. In the extremely rare instances where adult nematodes have been found in the tissues of a host, the larval forms found in that host's blood stream were, with only a few exceptions, automatically assumed to be of the same species. This is not necessarily the case. Among the exceptions in birds, a very few times a microfilaria, similar to that found in the blood, has been discovered and identified within the adult female nematode in the tissues of the same host. They were both then very properly considered as belonging to the same species.

The speaker, being familiar with Faust's work on human microfilariae, has followed his same methods of measurement in her studies of avian microfilariae, during which heart blood samples have been taken and studied from over 1,600 birds. Some extremely interesting results have been encountered. In one series of measurements, the microfilariae of four groups of birds, each of a different genus, were being compared, namely the derby flycatcher (*Pitangus sulphuratus*), the least flycatcher (*Empidonax minimus*), the crimson-collared grosbeak (*Rhodothraupis celaeno*), and the indigo bunting (*Passerina cyanea*). The relative percentage distances of the microfilariae in these four hosts were, respectively, to the nerve ring, 23.0, 23.3, 20.2 and 20.6; to the excretory pore, 36.5, 34.7, 32.1 and 31.9; to the first genital cell, 57.2, 58.3, 55.2 and 57.1; to the anal pore, 83.3, 82.3, 80.1 and 82.7. The overall measurements were as follows: derby fly-

catcher, overall length, 116.6 to 127.2 μ with an average of 121.9 μ , overall width, 4.2 to 5.3 μ ; in the least flycatcher, overall length, 119.7 to 130.3 μ with an average of 123.7 μ , overall width 4.2 μ ; in the crimson-collared grosbeak, 102.8 to 108.1 μ for overall length with an average of 106.6 μ , overall width 5.3 μ ; and in the indigo bunting, overall length 95.0 to 106.0 μ with an average of 102.6 μ , overall width 5.3 μ .

These figures are all so similar that, following Faust, it would be difficult to do other than to consider them all the same species of microfilaria, as there were no significant differences between the groups. It was then decided to carry out an analysis of variance with F values determined, according to George Snedecor in his book *Statistical Methods*. The results of this showed clearly that one population of microfilariae was present in the two flycatcher genera, and another different population of microfilariae in the indigo bunting-crimson collared grosbeak genera. It is therefore necessary to draw conclusions with caution from data obtained only from measurements made by means of Faust's method.

The statistical division of the microfilariae into populations also showed that the various curved, highly twisted or straight positions of the larvae, and their variations in the shape of body ends from round, bluntly tapered or sharply pointed, are not valid characteristics by which to separate species, as has commonly been done. All three body positions, and variations in end shapes, were found within a single population group.

Recently another study has been completed by the speaker of the microfilariae present in four quite closely related genera of birds. Examinations were made of heart blood smears of 58 western white-winged doves (*Zenaida asiatica mearnsi*), 14 eastern white-winged doves (*Zenaida a. asiatica*), 38 western mourning doves (*Zenaidura macroura marginella*) and 31 red-billed pigeons (*Columba f. flavirostris*). Following Faust, measurements of overall length and width, and from the anterior ends to the beginning of each of the four major internal landmarks, were made of what appeared to be nine different microfilarial forms. The percentage distances were similar enough in some cases, so that, in order to differentiate the populations accurately, it was necessary to treat the data statistically. F tests were run on every possible combination of the nine tentative forms, using all four internal

landmark measurements, according to an analysis of variance from Snedecor. These were examined in the light of Faust's statement of species differentiation whereby no two different species can have all four relative percentage distances alike. Of course, therefore, only one relative percentage distance to an internal landmark need differ between two species. The statistical probability resulted that there were eight separate populations present.

Of these, four populations were found in a total of 28 western white-winged doves, two populations in a total of 5 western mourning doves, and two populations in a total of 7 red-billed pigeons. One of the two microfilarial populations found in 2 of the western mourning doves was also present in 1 eastern white-winged dove. Double infections with two microfilarial populations were found in 6 western white-winged doves.

The microfilariae encountered in both the eastern white-winged dove and western mourning dove, and found statistically to represent a single population, consisted of two types of larvae that under the microscope would never have been considered identical. In one host they averaged $126.4\ \mu$ in length and all were $3.2\ \mu$ wide. They were highly twisted, and stained dark red with Giemsa, with densely massed nuclei. No space was found between the last of the posterior nuclei and the body end. In the other host they averaged $152.7\ \mu$ in length and all were $4.2\ \mu$ wide. They were straight in position, stained light pink with Giemsa, and their nuclei were scanty in number and loosely arranged. The posterior 5 percent of the body was usually free of nuclei. However, their measurements to all four internal landmarks, in terms of the relative percentage of body length, were statistically identical. Does this mean that a given microfilaria species may change in structure and appearance, perhaps in response to its parasitism of differing hosts? If this is so, whatever the reason, the problem of accurately separating one species from another becomes difficult in the extreme.

The finding of four separate microfilarial populations or species within a single host species, following Faust's method, complicates still further the taxonomy of this group. It means that no adult nematode may safely be designated the species to which a given microfilaria belongs, simply because both are found in the same individual at

the same time, as many workers have done in the past. Several more than one species may be present, and the problem of accurately establishing which microfilaria is produced by which nematode presents itself. If laboratory inoculation is possible and a life history may be followed, valid relationships may be obtained. Another method is to dissect from the adult female nematode within the host's tissues a microfilaria ready to be discharged into the blood stream, and to compare it with those already found in the blood. However, within many of the lower animal groups, the adult female nematodes are but rarely encountered, even after the most careful search, and in the majority of cases they are not available for examination.

It seems to the speaker that it would be most desirable for a number of other workers, interested in nematodes and their larval forms, to also try to carry out systematic tests of Faust's premise, to see whether, in fact, species differentiation may be based upon this series of measurements. It will necessitate the making of large numbers of what are undoubtedly tedious and time-consuming statistical analyses of variation of the data obtained. The most logical place to make the tests would seem to be in the field of tropical medicine with human microfilariae. Here, in many infections, the adult female nematodes may commonly be recovered, and a study of their larval forms may consistently be made. The speaker hopes that others will be interested in carrying out such investigations.

SUMMARY

The differentiation of microfilariae into separate species rests, at the present time, upon data presented by Earnest Faust in his book *Human Helminthology*, in 1949. He made a series of measurements from the anterior end of a microfilaria to the beginning of each of its four major internal landmarks, namely the nerve ring, excretory pore, first genital cell and anal pore. These distances he expressed in terms of their relative percentage of the total length of the larva and said that they, together with the size and relation of its length to breadth, were utilized in specific diagnosis since they were constant in the same species.

The speaker's work on microfilariae from the heart blood smears of over 1,600 wild birds leads her to urge other workers to make statistical analyses of variation of these internal percentage measurements, such as she has done, in order to test thoroughly the validity of these measurements in species separation. She

has found as many as four microfilarial populations (species) within a single host species, if this method is valid. No adult nematode, then, may safely be designated the species to which a microfilaria belongs, simply because both are found in the same individual. She has also found within a single microfilarial species such varying conditions as position of larva straight, curved or highly twisted; body ends pointed or round; nuclei extending entirely to or not entirely to the body ends; and nuclei either large or small in size. These, therefore, are not valid characteristics by which to separate microfilarial species.

FILARIOSE ET PURPURA ANAPHYLACTOÏDE CUTANÉ

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F. FLAMENT

et

G. HELDERWEIRT

L'apparition de poussées aiguës de purpura est un symptôme commun à différentes affections qui peut être produit de diverses manières. Dans sa variété idiopathique simple, c'est-à-dire non thrombocytopénique, elle connaît une grande variété de causes, depuis des causes mécaniques — stase veineuse ou embolie —, nutritionnelles, toxiques, voire dégénératives. La ressemblance de ces poussées avec la maladie sérique et la relation possible avec un état allergique est suggérée par l'existence de poussées successives déclenchées par des réactogènes variés. Ce purpura simple, récidivant aisément mais peu grave, peut être héréditaire et familial, et atteint plutôt des enfants et des adultes jeunes.

Sa terminologie est des plus variées: péliose rhumatismale de Schönlein (1839) et purpura abdominal de Henoch (1874) sont certes les principales appellations. Purpura rhumatoïde bénin, purpura anaphylactoïde, angéite allergique, allergides nodulaires de Gougerot, erythema elevatum diutinum de Haber, microbides allergiques, voilà une série de vocables confirmant l'opinion d'Osler, qui estimait que le syndrome Schönlein-Henoch est une entité clinique avec des symptômes protéiformes. Ruiter (1954) et Miescher (1958) ont repris récemment la question de nomenclature et bon nombre d'autres syndromes cliniques semblent s'y rattacher: granulomatose de Wegener, périartérite noueuse superficielle, angéite systématique, etc. Ceci

démontre que le syndrome de Schönlein-Henoch n'a pas forcément le caractère symptomatique limitatif que certains y voient.

Le début est parfois aigu, s'accompagnant de fortes fièvres, de céphalées, de malaise, d'une gorge sensible, suivi ou non d'arthralgies. Le signe clinique constant est celui de l'apparition, parfois des jours, voire des semaines après la fin des symptômes généraux, d'un purpura à pétéchies papuleuses, épargnant la face et le tronc et prédominant de façon remarquablement constante et symétrique aux membres inférieurs.

La distribution anatomique des lésions entraîne une subdivision artificielle du syndrome en divers types. Des manifestations articulaires concomitantes font obligatoirement partie du syndrome de Schönlein. Des manifestations viscérales provoquant des crises douloureuses abdominales, dont le diagnostic est souvent postopératoire, de même que les irrégularités radiologiques de la muqueuse du grêle font partie intégrante du syndrome de Henoch. Les signes cutanés accompagnant l'éruption peuvent être urticariens, vésiculaires, bulbeux, voire ulcératifs.

Le contrôle du sang qu'impose la présence d'hémorragies conduit aisément au diagnostic: un nombre de plaquettes, un temps de saignement et de coagulation, des taux de prothrombine et des autres facteurs de la coagulation normaux éliminent le purpura idiopathique thrombocytopénique de Werhlof.

La cause du syndrome est souvent inconnue, mais sa relation avec un état allergique est suggérée par les poussées épisodiques qui peuvent être déclenchées par des réactogènes variés. Toutefois, le déclenchement par divers produits tels les insecticides, les herbicides, les sulfonilamides, les antibiotiques, les barbituriques, les anticonvulsivants, sérums et protéines étrangères, est bien connu.

Les travaux d'Israël (1956) en font une maladie par iso-immunisation: l'injection dans le derme du cobaye de sérum de lapin anti-endothélium de cobaye déclenche un syndrome hémorragique non thrombocytopénique. Cette même réaction se produisant aussi par l'injection de sérum de malades atteints de glomérulonéphrite ou de syndrome de Schönlein-Henoch.

Le pouvoir capillaro-toxique des allergènes apparaît dans des études histopathologiques qui montrent que la réaction primaire est

celle d'une péricapillarite diapéditique au niveau des lésions. Duperrat (1950) a pu démontrer que le purpura anaphylactoïde expérimental et l'angéite allergique ont la même image histologique. Lorsque les lésions deviennent plus chroniques, on voit apparaître des granulomes monocytiques ou histiocytaires. Cette granulomatose cutanée allergique est l'équivalent de la granulomatose généralisée de Wegener et de l'artérite temporale, comme certaines lésions artériolaires décrites par Lewitt se sont révélées du type périartérite noueuse. Toutes ces formes chroniques sont sujettes à poussées aiguës avec nouvelle extravasation par perméabilité accrue des capillaires atteints.

La base du syndrome de Schönlein-Henoch serait donc une réaction non spécifique aboutissant à la fabrication d'anticorps anticapillaires. Leur apparition est liée à l'emploi ou l'introduction de substances variées: iode, quinine, belladone, bismuth, mercure, phénacétine, acide salicylique, chlorates, sulfones, diphénylhydrazine, benadyl, vaccin (influenza). On peut y joindre certains aliments, tels le poisson et le mouton et les allergènes bactériens (streptocoque), Gavidres (1948).

Une série d'observations de purpura vasculaire aigu apparaissant au cours de la filariose, probablement à loa-loa, nous amène à poser la question du rôle étiologique possible de l'allergène filarien, celui de la carbamazépine, ou de l'association des deux. La rareté relative du purpura S-H et de la filariose permet d'exclure toute coïncidence.

CAS I

Un enfant de 5 ans arrive à la clinique, 3 jours après qu'il a présenté un gonflement aux pieds et aux mains et 2 jours après l'apparition d'une éruption cutanée hémorragique aux chevilles, d'abord discrète, pour s'étendre progressivement aux deux membres inférieurs. Il est pratiquement afebrile (temp. rectale 37,5°C) et le restera pendant la durée de sa maladie. Les ganglions inguinaux, cervicaux et axillaires sont légèrement gonflés. Il n'y a pas de rate palpable, mais à la percussion elle atteint le rebord costal.

Les examens de laboratoire donnent les résultats suivants:

Hématologie	11-6	14-6	21-6	20-7	10-8	À 1 an
Globules blancs	23.400	28.500	16.600	21.100	12.100	11.800
Eosinos	35	61	36,5	38,5	—	3,5
Neutros non segmentés. . .	—	—	5	0,5	—	3,5
Neutros segmentés. . . .	44	19,5	43,5	34	—	4,5
Lymphos.	18	17,5	15	24	—	44,5
Monos.	3	2	—	2,5	—	3
Sédimentation	40	67,5	—	—	—	—
Plaquettes	44.000 (?)	483.000	262.000	317.850	390.600	—
Temps de saignement . . .	2'03''	1'45''	—	—	—	—
Temps de coagulation . . .	4'	1'30''	—	—	—	—
Rétraction du caillot. . . .	+	+	+	+	—	—
Temps de prothrombine . .	+ 100 %	—	—	—	—	—
Globules rouges	4.300.000	4.650.000	—	3.900.000	4.200.000	—
Hémoglobine.	71 %	45,6 g	—	14 g	—	—
Valeurs globulaires. . . .	—	0,99	—	1,05	1,01	—
Examen chimique du sang:						
Glucose	—	102 mg %	—	—	—	—
Urée.	—	25 mg %	—	—	—	—
Cholestérol	—	198 mg %	—	—	—	—
Protéines totales.	—	9,85 g	—	—	—	—
Alb.	—	39,1 %	—	—	—	—
α_1	—	3,6 %	—	—	—	—
α_2	—	10,2 %	—	—	—	—
β	—	9,4 %	—	—	—	—
γ	—	37,7 %	—	—	—	—
Thymol	—	8,2	—	—	—	—
ZnSO ₄	—	21,5	—	—	—	—
H ₂ O.	—	19	—	—	—	—
CdSO ₄	—	++	—	—	—	—
Lugol	—	+++	—	—	—	—

Centrifugation: *A. perstans*. Urines: rien à signaler. Selles: négatif.

Cet enfant avait dans ses commémoratifs une symptomatologie de loase; toutefois, on n'a trouvé dans le sang circulant que la filaire banale *A. perstans*: cette constatation est usuelle.

Le diagnostic était évident: purpura rhumatoïde non thrombopénique. L'origine suspectée est la filariose à loa-loa, dont l'importance comme allergène est bien connue.

Un traitement classique à la carbamazine fut fait sans incident. Une semaine après le début du traitement, les taches purpuriques ont disparu en laissant des croûtes, comme après des nécroses cutanées: ceci est inusuel pour un Schönlein.

Il n'y a pas eu de rechute, et l'enfant se porte parfaitement depuis. Par contre, sa mère et un petit frère ont présenté par la suite un syndrome similaire (cas VI et VII).

CAS II

Un mois plus tard, est amenée à la clinique une fillette de 5 ans qui présentait au Maniema (C. B.), depuis plusieurs mois, un purpura qui aurait été accompagné de fièvre, de splénomégalie, d'une rate palpable, d'une diminution du nombre de plaquettes (chiffre non renseigné) et d'une irrétractabilité du caillot. Le diagnostic posé est celui de maladie de Werlhoff. L'enfant n'a jamais eu la moindre hémorragie.

À l'anamnèse, la longue durée de l'affection fut confirmée et on apprend que les éruptions purpuriques surviennent en poussées environ tous les 28 jours.

Peu avant le départ d'Afrique, l'enfant montre un œdème palpébral de l'œil gauche, attribué au passage d'une filaire loa-loa.

Les examens de laboratoire fournissent les résultats suivants:

Hématologie	28-7	27-9
Globules blancs	7.800	9.200
Eosinos	52	55
Basos	0,5	—
Neutros non segmentés.	—	—
Neutros segmentés.	15,5	13
Lymphos	31	30
Monos.	1	2
Sédimentation	1	9
Plaquettes	204.700	339.450
Temps de saignement	3'32''	1'15''
Temps de coagulation	3'	4'15''
Rétraction du caillot.	+	+
Globules rouges	4.600.000	4.650.000
Hémoglobine.	92,2 % -15,7 g %	94 % -16 g %
Valeurs globulaires	1	1,20
Centrifugation	<i>A. perstans</i>	—
Scarification	<i>A. perstans</i>	—
Examen chimique:		
Glucose	89 mg %	—
Urée.	22 mg %	—
Cholestérol.	210 mg %	—
Protéines totales	770 g	—
Thymol	2,83	—
ZnSO ₄	5,77	—
H ₂ O.	3,64	—
CaSO ₄	Nég.	—
Lugol	Nég.	—
Céph. cholestérol.	+	—

Urines: rien à noter. Sells: négatif.

Un traitement antifilarien classique a été administré: cette thérapeutique n'a entraîné aucune complication. Le purpura a disparu et la fillette se porte bien.

CAS III

Un an plus tard, une autre fillette, âgée de 9 ans, nous est envoyée par les Drs. A. Musin et G. Bruynseels. Dans les antécédents on ne note rien de particulier. Enrhumée il y a une semaine, elle développe, deux jours après le début de ce rhume, des taches et des nodules purpuriques au niveau des deux pieds, des chevilles et des jambes. Elle n'a présenté aucune température, ni de douleurs ou de gonflement articulaire. Cette enfant ne paraît pas malade et ne se plaint de rien.

L'éruption consiste en taches et nodules purpuriques: les taches ressemblent à celles qu'on voit dans le purpura rhumatoïde de Schönlein, seulement il manque les douleurs articulaires. D'autre part, des nodules purpuriques ne sont pas classiques dans le Schönlein.

Une biopsie, prélevée par le Dr. Laloux et examinée par le Prof. L. van der Meiren, montre «une image très typique de capillarite aiguë. Les parois des vaisseaux sont désintégrées, les lumières vasculaires sont bourrées de polynucléaires eosinophiles. L'épiderme est infiltré de polynucléaires et montre en certains points un processus de phlycténisation». Il n'y a pas de signes de spécificité.

Les examens de laboratoire (Dr. A. Musin) apportent ce qui suit:

Hématologie	24-10	26-10	29-10
Globules blancs	59.600	40.300	—
Eosinos	66	55	—
Neutros	11	21	—
Basos	—	—	—
Lymphos	22	18	—
Monos	—	6	—
Sédimentation	22	—	—
Plaquettes	—	476.000	—
Temps de saignement	2'30''	—	—
Temps de coagulation	5'	—	—
Temps de prothrombine	100 %	—	—
Signe du lacet	Nég.	—	—
Rétraction du caillot	+	—	—
Globules rouges	4.760	4.860	—
Hémoglobine	40,7 % - 14 g %	—	—
Valeurs globulaires	0,95	—	—
Centrifugation	—	▼ —	<i>A. perstans</i>
Scarification	<i>A. perstans</i>	—	—

Urines: négatif. Selles: *Trichiuris trichiura*, peu nombreux.

Un traitement antifilarien a été entrepris. Tout est rentré définitivement en ordre.

CAS IV

Madame C., 29 ans, consulte en 11/56 pour œdèmes malléolaires avec arthralgies. On relève dans les antécédents un séjour de 3 ans au Congo Belge sans maladie tropicale. Le mari y a été atteint de filariose.

À l'examen: fluxion articulaire au niveau des chevilles, purpura papulaire symétrique au niveau des pieds, des jambes, sans température. Impotence fonctionnelle importante, cédant au repos. Une récurrence survient 2 mois après, alors que sont parvenus les examens suivants:

Hématologie :

Globules blancs.	35.000
Eosinophiles.	22 %
Polynucléaires.	55 %
Sédimentation.	20 mm
Temps de prothrombine.	100 %
Plaquettes.	286.000
Temps de saignement.	2'
Temps de coagulation.	7'
Rétraction du caillot.	Normale
Hanger, thymol.	N.
Examen des urines.	N.

Poussée de grande eosinophilie, leucocytose de 77.600 blancs et pourcentage fluctuant des eosinophiles (54 %, 68 %, 79 %) s'expliquent par la découverte de *A. perstans*. En 4/57 et 7/57 récurrence: la malade, invitée à se présenter au moindre symptôme, est atteinte cette fois, outre le PSH, d'une éruption morbilliforme prurigineuse avec gonflements migrants. Pas de signe de lacet. État général florissant. Un traitement au delta-cortisone entre les poussées fait chuter l'image à 13.200 blancs et 16 % d'eosinophiles. Un traitement à la carbilazine est entrepris. La récurrence du PSH détermine l'administration simultanée de delta-cortisone. La patiente omet le second produit à l'issue de la deuxième cure et présente le 19/9/57 une nouvelle récurrence de purpura.

Trois cures ultérieures seront poursuivies sans incidents. Il n'y a plus eu de récurrence depuis la guérison de la filariose (fin de la cinquième cure fin janvier 1958). Soit une première cure en juillet,

sous couverture de corticoïdes, fort bien supportée, sans la moindre poussée purpurique, une seconde cure mi-août, où l'intéressée omet le prednisone et développe une poussée purpurique aux jambes avec température, qui guérit en 4 jours par la cortisone, la troisième cure en octobre avec prednisone sans incidents, de même que les quatrième et cinquième.

Hématologie	2-8-57	19-11-57	13-6-58
Globules blancs	10.700	38.800	14.850
Eosinos	24 5	68	48
Basos	1	—	—
Neutros non segmentés.	2,5	12	1,5
Neutros segmentés.	36	19	32
Lymphos.	36	1	17,5
Monos.	—	—	1
Sédimentation	9	16,7	15,5
Plaquettes	266.800	231.000	260.150
Temps de saignement	4'	2'30''	3'16''
Temps de coagulation	3'30''	1'45''	4'36''
Rétraction du caillot.	+	+	+
Temps de prothrombine.	(17'') 40 %	—	—
Centrifugation	Nég.	<i>A. perstans</i> (2/1)	<i>A. perstans</i> (1/1)
Globules rouges	4.600.000	4.200.000	4.300.000
Hémoglobine.	100% (17 g %)	85,5 % (14,5 g %)	88,5 % (15 g %)
Valeurs globulaires.	1,09	1,01	1,02
Examen biochimique:			
Urée.	29	29	28
Cholestérol.	256	232	290
Phosphatase alcaline.	9	13,5	7
Protéines totales.	8,47	8,19	8,85
Alb.	56,6	55,8	54,3
α_1	5,5	3,9	4,7
α_2	7,6	9,1	9,4
β	11,0	11,3	12,7
γ	19,3	19,9	18,9
Thymol	7	7,6	5,4
ZnSO ₄	10,1	17,2	10,1
H ₂ O.	10,1	13,0	8,6
CdSO ₄	+	++	+
Lugol	0	+	0
Hanger	0	++++	+++

Urines : négatif. Selles : négatif.

CAS V

Une jeune fille atteinte de loase assez importante est mise en traitement à la carbamazine. Au deuxième jour du traitement, elle fait un accès fébrile, qui se complique d'une éruption maculopapuleuse

purpurique des deux membres inférieurs. L'administration de corticoïdes a rapidement raison de cette réaction. La poursuite du traitement se fait sans incidents.

Hématologie	15-6	17-7
Globules blancs	12.600	45.800
Eosinos	47,5	56
Basos	0,5	—
Neutros non segmentés.	1	6
Neutros segmentés.	34	22
Lymphos	16	14
Monos.	1	2
Sédimentation	10	18
Plaquettes	—	254.300
Temps de saignement	—	2'32"
Temps de coagulation	—	3'
Rétraction du caillot.	—	+
Temps de prothrombine	—	+ 100 %
Centrifugation	Mph. <i>loa-loa</i> + Mph. <i>perstans</i>	Mph. <i>perstans</i> —
Exame biochimique :		
Urée.	25	26
Cholestérol	165	182
Phosph. alc.	12	15
Protéines totales	8,22	8,63
Alb.	—	45,8
α_1	—	6,2
α_2	—	11,2
β	—	12,7
γ	—	27,8
Thymol	12,6	16,3
ZnSO ₄	19,3	18,2
CdSO ₄	++	++
H ₂ O.	22	17,8
Lugol	+++	++
Hanger	++++	++++

CAS VI ET VII

L'intérêt de ces deux dernières observations réside dans le fait qu'il s'agit de la mère et du petit frère du cas I. La mère est filarienne reconnue depuis 7 ans, mais présente depuis un an et demi des poussées des lésions papuleuses purpuriques, sans autres signes. L'infestation est probablement mixte à *loa-loa* et *A. perstans*. Elle a présenté des œdèmes de Calabar et, lors du traitement, des filaires adultes sont apparues sous la peau en différents endroits.

Les examens de laboratoire fournissent les données suivantes:

Hématologie	31-7-51	9-4-58	28-5-58	18-7-58
Globules blancs	10.300	15.000	6.950	11.400
Eosinos	55	41,5	38	16
Basos	0,5	—	—	—
Neutros non segmentés . .	—	—	—	1
Neutros segmentés	28	16	35	58
Lymphos	15	39	26	22
Monos	1,5	3,5	1	3
Sédimentation	15,5	16	9	—
Plaquettes	—	166.500	183.025	124.800
Temps de saignement	—	3'30"	3'30"	3'17"
Temps de coagulation	—	2'	30"	1'26"
Rétraction du caillot	—	+	+	+
Temps de prothrombine . . .	—	35 %	30 %	36 %
Centrifugation	—	<i>A. perstans</i>	<i>A. perstans</i>	Nég.
Globules rouges	4.146.000	3.950.000	4.050.000	3.750.000
Hémoglobine	80,9 % (12,8 g %)	73,5 % (12,5 g %)	79,5 % (13,5 g %)	—
Valeurs globulaires	0,98	0,94	0,99	—
Examen biochimique:				
Urée	44	—	29	—
Glucose	—	—	81	—
Cholestérol	240	—	246	—
Phosphatase alcaline	—	—	9,5	—
Calcium	—	—	9,6	—
Protéines totales	8,31	—	8,24	—
Thymol	2,85	—	2,00	—
ZnSO ₄	7,27	—	9,3	—
H ₂ O	—	—	4,12	—
Lugol	—	—	0	—
Hanger	—	—	0	—

Urines: négatif. Selles: négatif. Suc dermic (scarification): *A. perstans*.

Suite au traitement antifilarien fait sous couverture de prednisolone, il n'y a eu aucun incident. Le taux de prothrombine est dans ce cas plutôt bas et il y avait une insuffisance de vitamine C. Un traitement complémentaire adéquat a été instauré simultanément. Le syndrome purpurique n'a plus réapparu.

Le frère, âgé de 5 ans, a été hospitalisé au décours d'une éruption papuleuse purpurique, dont on observe les séquelles. En outre, il y a une dermite papuleuse extrêmement prurigineuse et non purpurique aux membres supérieurs et inférieurs, ainsi que sur le thorax.

L'état général de l'enfant est excellent et l'examen clinique est négatif. Le lendemain de son hospitalisation et avant tout traitement, il fait un fort accès fébrile avec engorgement débutant des parotides: il fait les oreillons.

Dès le retour à la normale, un traitement spécifique est entrepris: il provoque un œdème de Calabar typique et une exacerbation de la dermatose.

Protocole des examens de laboratoire:

Hématologie	28-5-58
Globules blancs	12.100
Eosinos	55,5
Basos	—
Neutros non segmentés	—
Neutros segmentés	19
Lymphos.	25,5
Monos	—
Sédimentation	6,5
Plaquettes	171.600
Temps de saignement	3'45''
Temps de coagulation	1'30''
Rétraction du caillot	+
Temps de prothrombine	34 %
Globules rouges	3.900.000
Hémoglobine	76,5 % (13 g %)
Valeurs globulaires.	0,98
Examen biochimique:	
Glucose	81 mg %
Urée.	23 mg %
Cholestérol.	200 mg %
Calcium	10,8 mg %
Protéines totales	7,81 % g
Alb.	45,7
α_1	6,1
α_2	10,7
β	15,2
γ	27,3
Thymol	3,20
ZnSO ₄	9,1
CdSO ₄	+
H ₂ O	4,29
Hanger	0
Lugol	0

Urines: normales. Selles: négatives. Scarification cutanée; *D. perstans*.

L'évolution subséquente est sans incidents.

DISCUSSION

Il s'agit d'une association pathologique non encore décrite, et, somme toute, assez rare, puisque nous n'avons observé que 7 cas

possibles en 4 ans, dont 3 dans la même famille, pour 349 cas de filariose soignés pendant cette période. Il y a lieu de noter que pour les 4 années précédentes et 222 cas observés et traités, cette symptomatologie était totalement absente. Elle est d'ailleurs suffisamment typique pour pouvoir affirmer qu'elle n'aurait pas échappé au patient, au cas où le médecin ne l'aurait pas recherchée ou vue.

Connaissant la valeur aggressive de l'allergène filarien, une telle possibilité aurait pu être suspectée depuis longtemps, vu l'extraordinaire variété des causes du purpura de Schönlein-Henoch. C'est en outre une nouvelle confirmation que la symptomatologie propre de la filariose est reproduite fidèlement par le traitement spécifique et qu'elle peut être en quelque sorte complétée de manière iatrogène.

L'apparition des symptômes de S-H en l'absence de toute intervention thérapeutique (cas I, II, III, IV, VI, VII) montre que l'allergène filarien peut être l'agresseur par lui-même. Les réactions violentes au cours d'un traitement à la carbamazine sont dès lors à rattacher à la libération brutale d'une forte quantité d'allergènes, plutôt qu'à l'action de la carbamazine elle-même. C'est d'ailleurs un fait actuellement bien connu que le traitement des filarioses fait réapparaître dans sa phase initiale du moins la symptomatologie propre de la parasitose.

La démonstration quasi expérimentale par la carbamazine (cas IV) de la récurrence du purpura anaphylactoïde, suite à l'omission volontaire du corticoïde protecteur, montre l'action directe de l'allergène filarien, car l'administration de carbamazine sans corticoïde dans la phase tardive de la cure ne produit aucune réaction.

L'analyse détaillée des 7 cas rapportés montre en tout premier lieu que les cas II et VI ne sont guère typiques, vu leur chronicité et un soupçon qu'il pourrait s'agir plutôt de variétés thrombocytopéniques. Cette chronicité méritait d'être signalée. En outre, ces cas atypiques sont un indice qu'outre le purpura anaphylactoïde cutané la filariose est capable de produire d'autres symptômes. Ainsi semble-t-il logique de s'attendre à des symptomatologies abdominales. On ne peut en outre pas écarter la possibilité d'une auto-aggression anti-thrombocytes, qui demande une recherche approfondie de tels anticorps spécifiques d'origine médicamenteuse.

Au point de vue symptomatologie, il y a lieu de retenir que l'état général des patients est généralement fort bon, sauf deux: ils sont

afébriles, et n'ont aucune symptomatologie autre que locale. Deux sont porteurs de ganglions, ce qui est banal dans la grande eosinophilie, et deux ont montré de l'œdème. L'éruption aux membres inférieurs est dans 5 cas papulaire, voire nodulaire, deux patients montrent en outre une certaine vésiculation qui se nécrose; sauf deux, ils ont tous des taches purpuriques simples. Deux cas ont en outre une éruption généralisée prurigineuse, signature fréquente de la filariose.

Six ont plus de 10.000 globules blancs (quelques chiffres tels 28.500, 35.000, 53.600, 40.300, 38.800) et tous plus de 50 % d'eosinophiles (soit dans l'ordre les maxima suivants: 61, 52, 66, 79, 68, 72, 55 et 55,5 %), c'est-à-dire avec grande eosinophilie. La sédimentation est généralement moyennement accélérée (5/7), les plaquettes sanguines sont normales (5/7), les temps de saignement et de coagulation sont normaux (7/7), le taux de prothrombine aux environs de 100 % (5/7), les γ -globulines élevées (37,7 % — 19,3 — 19,9 — 18,9 — 27,8 — 27,3 %).

Le point le plus délicat est la présence constante de *D. perstans*, filaire notoirement inoffensive et ne subissant aucun effet de la chimiothérapie actuelle, s'opposant aux constatations cliniques qui sont celles de la loase. Cette observation n'est pas nouvelle, bien au contraire, et à titre exemplatif, nous faisons suivre les résultats de la recherche des microfilaries par double centrifugation de sang citraté dans des cas cliniquement probables de loase.

	Nombre d'examens	<i>D. perstans</i>	<i>Loa loa</i>	<i>Loa loa</i> + <i>perstans</i>
1955.	126	24	7	1
1956.	205	54	6	3
1957.	227	51	3	6
1958 (6 mois)	114	39	9	3
	672	168	25	13

Ceci donne donc 38 examens positifs pour loa-loa, contre 181 résultats positifs pour *D. perstans*, sur les 672 cas suspects cliniquement de loase, soit un coefficient de 4,8 en faveur de la microfilaire *perstans*, ce qui correspond à notre propre série avec 1 cas sur 7. Malgré tout, on ne peut pas s'empêcher d'être troublé par cette discordance entre la clinique et les résultats de laboratoire. Certes,

on dit que les microfilaires de loa-loa sont plus rares dans le sang. Affirmation qui est infirmée par la découverte non rare de grands nombres de microfilaires de loa-loa chez certains et par une observation, où, non traitée, une patiente montre régulièrement plus de 100 microfilaires loa-loa par lame.

Ceci nous amène à poser la question s'il n'y a pas des infections humaines par d'autres filaires, qui tout en ne devenant pas matures dans l'organisme humain, n'en produisent pas moins une symptomatologie type loa-loa, c'est-à-dire œdème de Calabar, œdème de la paupière, passage de filaires par la conjonctive oculaire, apparition sous la peau et sensibles à la carbamazine.

Nous posons la question sans pouvoir y répondre, en songeant à certaines filaires des genres *dirofilaria* et *noctiella*, qui ont déjà été trouvées dans les tissus conjonctifs de l'homme en Floride, en Thaïlande, en Méditerranée Orientale, en Argentine et en Afrique (Faust). La réponse sera fournie par l'examen systématique des filaires extraites de la conjonctive oculaire ou de la peau, qui ont toujours été classées sans autre forme de procès comme devant être des filaires loa-loa.

RÉSUMÉ

L'observation d'une éruption cutanée purpurique chez 7 patients atteints de filariose amène les auteurs à conclure à une relation étiologique entre le purpura anaphylactoïde cutané, type Schönlein, et cette parasitose. La symptomatologie, qui se caractérise par l'irrégularité de manifestations articulaires, la nature plutôt papuleuse de la lésion purpurique et peut-être une certaine tendance à la chronicité, est décrite en détails. Les données de laboratoire sont fournies in extenso.

Une question relative à la nature propre de l'agent étiologique est posée, mais laissée sans réponse objective.

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GENERAL DISCUSSION

PROF. A. W. WOODRUFF (U. K.) — Dr. Janssens observations on purpura in loiasis are of great interest although some caution is necessary before the association can be accepted as proved.

In the Medical Unit at the Hospital for Tropical Diseases, London, we have been looking for purpura in filarial infections ever since, some years ago, we had a patient with severe purpura and onchocerciasis, but during the last 10 years we have had over 150 patients with onchocerciasis and 250 with loiasis and I have seen no further purpura in these patients.

Many cases of purpura were reported among British troops in North Africa during the last war and no cause could be found. It seems therefore that at some times and in some places, in warm climates, purpura, unassociated with any known cause, may become not uncommon, and this makes it difficult to associate it causally with filariasis until figures are produced which, when subjected to statistical analysis, make it clear that chance can be excluded.

DR. JEAN SCHNEIDER (France) — À l'appui de ce que vient de dire le Prof. Woodruff, j'estime que le purpura peut être une coïncidence. J'ai traité plus de 500 malades atteints de filariose à f. loa et le seul cas de purpura que j'ai observé est celui d'un malade qui avait déjà eu un purpura plusieurs années avant de contracter la filariose — il y avait donc une prédisposition certaine; j'ai remarqué que les 3 cas sur 7 de la communication appartenaient à la même famille, ce qui est un argument de plus en faveur d'une prédisposition de l'individu; bien entendu, la filariose peut être un facteur déclanchant du purpura chez un sujet prédisposé.

DR. JEAN SCHNEIDER (France) — Je suis étonné de voir que la filariose de laboratoire présentée par Baltazard au Congrès d'Istanbul en 1953 n'ait pas retenu l'attention de nombreux chercheurs. Grâce à cette filariose (*D. vite*) transmise à des merions par des ornitho-

dores, j'ai pu en 1956, après échec d'essais de traitement par l'Hétra-zan et la Suramin, démontrer l'activité antifilarienne du TWSb de Friedheim. Ce premier essai m'a permis ultérieurement de traiter efficacement des singes spontanément infestés par *D. vanhoofi* et ces recherches ont été le point de départ du traitement de l'onchocercose humaine par le TWSb.

DR. H. DE ROOK (Netherlands) — In reply to the remarks of Dr. Kessel I should like to give some details about the method on which I have proved that *M. uniformis* is a carrier of the periodic *W. bancrofti* in Netherlands New Guinea.

In the first place, wild mosquitoes were caught during spontaneously feeding on a carrier in a house. Of 97 *M. uniformis* 87 specimens proved to be infected (experimental infection rate $\pm 90\%$). Of 16 specimens examined 12 $\frac{1}{2}$ days after the infective feed 14 showed mature larvae.

In the second place, 227 *M. uniformis* were caught in the houses in the morning; 45 of these mosquitoes were infected (all stages of developments) and 3 specimens showed mature larvae. The natural infection rate was 19.8 percent and for mature larvae 1.3 percent.

In another area of New Guinea van Dijk caught 18 *M. uniformis* on a carrier and of 12 of these complete development took place.

It must be concluded that *M. uniformis* in Netherlands New Guinea is a vector of *W. bancrofti*.

DR. P. G. JANSSENS (Belgique) — C'est avec grand plaisir que j'ai pu constater que ma communication m'a valu des interlocuteurs de qualité. J'en suis d'autant plus aise que je partage entièrement leur façon de voir quant à la prudence que commande l'interprétation de toute nouvelle symptomatologie en matière de loase.

Cette prudence ne nous dispense toutefois pas du devoir élémentaire du clinicien de rassembler les faits d'observation, de les analyser dans leur contexte et d'en dégager au moment voulu les conclusions qui s'imposent.

À titre d'exemple je mentionnerai le fait que malgré que nous ayons rassemblé un matériel plutôt abondant de cardiopathies rencontrées chez des loasiques, je ne me crois pas autorisé d'affirmer une relation de cause à effet vu l'absence de preuves objectives d'une étiologie filarienne.

Par contre, le purpura non thrombocytopénique idiopathique connaît à la fois un terrain qui peut être héréditaire et familial et des causes déclenchantes multiples et variées. Rien ne permet d'affirmer que parmi ces divers allergènes non spécifiques le filarien, dont l'aggressivité est bien connue, devrait être exclu.

Nous croyons d'ailleurs avoir apporté une évidence très satisfaisante, à défaut de preuve absolue, de l'existence d'un syndrome anaphylactoïde cutané lié à la filariose à loa-loa, dont la symptomatologie est quelque peu différente du Schönlein classique par son aspect nodulaire, qui le rapproche du strophulus infantile, par sa forte eosinophilie, par son hypergammaglobulinémie et son absence d'atteinte rénale. Cette évidence repose en ordre principal sur les poussées iatrogènes et le succès de la thérapeutique spécifique.

DR. JOHN KESSEL (U.S.A.) — It would appear from the previous papers that S.E. Asia, Indonesia and perhaps N.W. Melanesia constitute an area which might be termed the melting pot of filariasis, as much study in progress there is producing revolutionary results. Statements indicating that *Mansonia* may serve as vectors of *W. bancrofti* are rare. In trying to determine the origin of the statement in the literature that *M. juxtamansonia* and *M. pseudotitilans* are carriers of *W. bancrofti* in Brazil, I wrote to Dr. Rachou and received the following reply: «We cannot find the origin of the statement, and we believe if larvae have been found in these mosquitos, they probably were of other species of filariae.» I wonder if Dr. De Rook would be good enough to give more details concerning his observations on the transmission of *W. bancrofti* by *Mansonia* in New Guinea.

DR. HENRI GALLIARD (France) — Mr. Hawking nous a dit qu'il avait plusieurs souches d'*Aedes aegypti* dans son laboratoire, de provenance différente, et que leur réceptivité vis-à-vis de *Dirofilaria immitis* était très différente. Ainsi, en matière de transmission des filaires par les moustiques il faut tenir compte du facteur espèce ou race d'espèce de filaire, du facteur invertébré transmetteur et du facteur vertébré, homme ou animal, qui est individuel ou occasionnel.

À ce propos je voudrais rappeler les résultats d'expériences faites il y a plus de 20 ans au Tonkin avec *Wuchereria bancrofti*. Alors que tous les *Culex fatigans* s'infectaient facilement, j'avais observé que avec *Aedes aegypti* et *W. bancrofti* l'infection ne se produisait

que rarement. Il y avait des porteurs de microfilaires qui étaient de bons infecteurs et d'autres qui étaient mauvais.

En ce qui concerne la race du parasite, au Tonkin j'avais observé la très grande réceptivité d'*Aedes albopictus* vis-à-vis de *Dirofilaria immitis*. Or à la même époque on observait en France que ce même moustique était complètement réfractaire à une race de *D. immitis* au Sud de la France.

Il faut donc toujours tenir compte de ces facteurs et ils expliquent les opinions parfois divergentes des différents auteurs.

DR. OLLE GABINUS (Sweden) — Working with a new type of centrifuge, Spiralcentrifuge (Damielsson-Lundmark), I have found it very useful for concentration of microfilariae in the blood. It consists of two parts, an *electric motor* and two *plastic discs* with a diameter of 15 cm. In one of the discs is a spiral cut. Citrated blood (1 part 3,8 percent citrate and 4 parts blood) is sucked up in a very thin plastic tube. A knot is made in one end of the tube. The tube is placed in the spiral cut with the knot at the outer end. The tube is cut off at the inner end. The length of the tube will be about 45 cm. Then the other disc is used as a lid in order to hold the tube in the right position. The discs are fastened in a horizontally position with a screw on the vertical motoraxis. Then you centrifugate for 3 minutes (2,500 r/min.). You will find all the microfilariae in the layer of white blood corpuscles. In order to reach the microfilariae you cut off the tube where the leukocyte-layer is situated and press it out on a slide. A coverglass is put on and in the microscope you will see the microfilariae crawling around.

The point is that with this method the blood column is about 45 cm and all the microfilariae are concentrated to the layer of leucocytes; it is a column about 2 mm long where they are easy to reach.

During the last years I have observed some interesting features of the blood in cases of loiasis. In some of the cases with heavy microfilaraemia the eosinophils ran a parallel course with the microfilariae, that is, they have a maximum in the day and a minimum in the night. In cases with very few microfilariae there is no or very small fluctuations of the number of the eosinophils.

PAPERS OFFERED AS FREE COMMUNICATIONS TO THE
SUBSECTION 2—FILARIASIS BUT NOT READ AT THE
SESSIONS DUE TO THE ABSENCE OF THE AUTHORS

SOME FACTORS AFFECTING MICROFILARIAL DENSITY
AND PATHOGENICITY IN EXPERIMENTAL EPIDEMIO-
LOGY OF AN ANIMAL FILARIOID INFECTION

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The past decade has been one of interesting developments in the natural history of filarioid infections of man. Loiasis has been demonstrated in forest monkeys and drills of the Cameroons (1). *Wuchereria malayi* of man and *malayi*-type infections have been found in wild animals, cats and dogs in Malaya (2) and a *malayi*-type infection in cats and dogs in East Africa (3). Besides raising questions of zoonoses of human filarial diseases, these discoveries have opened the way to experimental filariasis with infections in animals parasitologically identical with, or closely similar to, a mosquito-borne filariasis and loiasis of man. These new materials have certain practical difficulties. Time factors, as in any filarioid infection, are long; monkeys, cats and dogs are troublesome to keep in numbers as laboratory animals; vector transmission to clean hosts is desirable, ultimately obligatory, for a true laboratory strain of the helminth, and, although this presents few obstacles for the mosquito-borne filariasis, laboratory culture of the tabanid vectors, *Chrysops* spp., of loiasis is still elusive. Despite these factors, remarkable progress has already been reported in experimental filariasis with two of these new filarioid systems (4, 5, 6). In view of the handicaps involved, it seems appropriate at

this early stage of new opportunities in this field to offer a general consideration of some work on the filarioid infection of the cotton rat. This rodent infection resembles, if comparison is permissible, *perstans*-infection of man in that the developing and adult worms inhabit the coelomic spaces of the mammalian host and the sheathed microfilariae are non-periodic in the blood stream. In passing, one might note that the vector is a blood-sucking mite and not a ceratopogonid midge. A good deal of long-term experimentation has been attempted with this filarioid system with results which suggest interesting phenomena to watch for as researches with these new systems, related to human infections, continue to expand.

SIMPLE INFECTIONS

It is well established for cotton rat filariasis that infective larvae mature to adult worms in the pleural spaces and, from there, microfilariae escape to the blood stream. If worms are transmitted on a single occasion only, the peak of the microfilaraemia is roughly proportional to the number of adult worms in the pleural cavities — but only for very light infections of up to about 12 to 20 (female) worms. With greater numbers, the microfilaraemia ceases to increase proportionately. This is represented by rats A, B and C (Fig. 1). However, when worms become very numerous in this primary site, the pleural cavity, their growth is inhibited. Some worms then occur in the peritoneal cavity, and in this secondary site growth is not, or much less, restricted (7). Thus, transmission of a large number of infective larvae on a single day to a cotton rat resulted, at autopsy 20 weeks later, in 301 females of 6.2 cm mean length and 255 males in the pleural spaces, but only 5 females of 11.25 cm mean length and 2 males in the peritoneal cavity. This case shows that deviation to a secondary site can result simply from overcrowding of the primary site. Other work (8, 9) indicates that a true immunological host-parasite reaction may also operate in reinfections.

REINFECTIONS

A first infection does not give complete immunity to reinfection. Indeed, unlimited permutations and combinations of frequency and

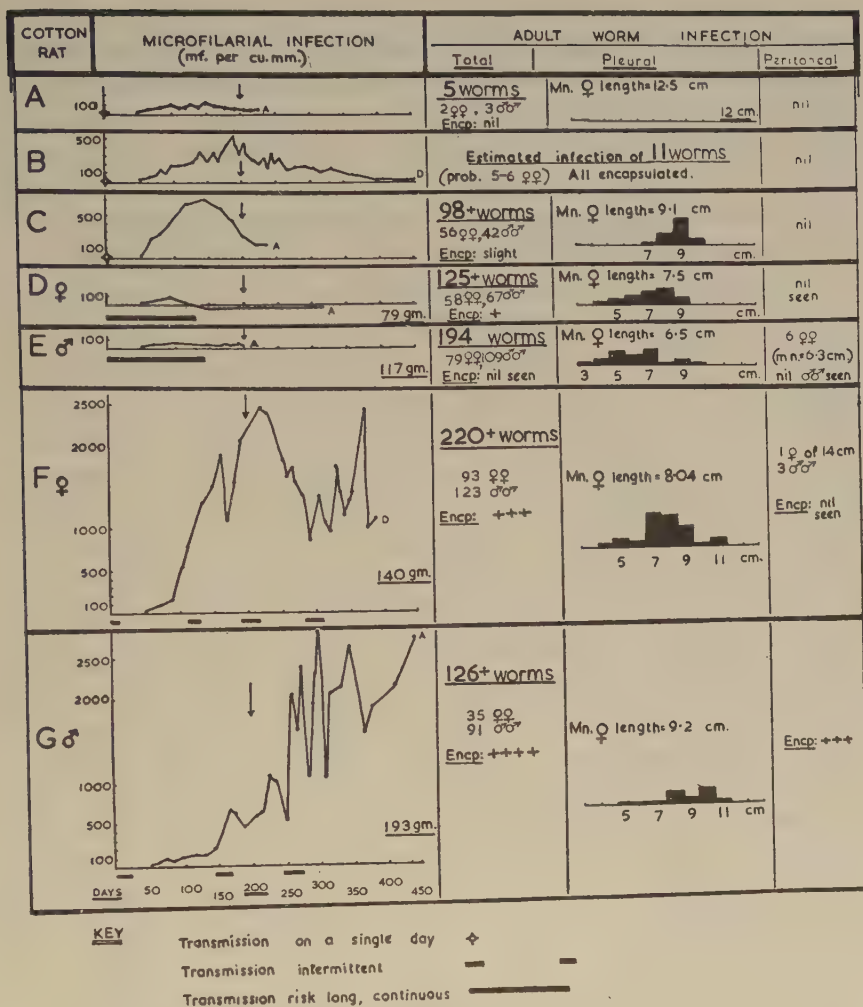


Fig. 1 — Illustrating for cotton rat filariasis effects on microfilaraemia, adult worm infection, and weight of host of continuous (rats D and E) and intermittent (rats F and G) exposure to reinfections. Rats A, B and C are infections following transmission on one single day of small (rat A) to moderate (rat C) numbers of worms

amount of reinfection are possible with, as a result, great variability in the course of the infection in the cotton rat. A comprehensive study of all the possibilities is impracticable, but markedly contrasting pat-

terns of reinfection have been studied (10). These are briefly presented and discussed in this paper. If transmission is markedly intermittent (rats F and G, Fig. 1), worms of successive transmission periods mature quite successfully and there is a prolonged high microfilaraemia. On the other hand, where the exposure to transmission is continuous over several months (rats D and E, Fig. 1) the blood may soon become and remain negative, or nearly so. The pleural worms in these rats are below normal size and produce few or no microfilariae. This alone could explain the low or negative microfilarial counts, although some rapid destruction of circulating microfilariae may also be operating (7). What happens to peritoneal worms in both these types of reinfections is not quite clear. There are indications (7) that they are, perhaps, fairly readily encapsulated under conditions of active transmission (whether of the intermittent or continuous type). Complete cessation of further transmission, however, leaves them free to mature to long, highly productive worms contributing substantially to the microfilaraemia (Fig. 2). It seems probable that peritoneal worms play an important part in producing the high microfilaraemias of intermittent infections, before succeeding transmissions initiate their encapsulation (rat G, Fig. 1). Intermediate levels and durations of microfilaraemias have been observed in other cotton rats, with indications of both being reduced as transmission periods became longer and closer together.

As regards pathogenicity: Cotton rats D and E, and others (7), were lethargic, of subnormal weight, hunched and with raised fur for several weeks before autopsy. Cotton rats F and G were never abnormal in appearance nor activity, and their weights were good at sacrifice.

DISCUSSION

These results suggest how in a filarioid infection in nature some degree of seasonal transmission and, in contrast, perennial transmission may affect differently the incidence (and density) of microfilaraemias and of filarial disease. If filarial disease is associated with developing or adult worms, its incidence in a population should be greatest with perennial transmission, and the microfilaraemia rate and microfilarial counts low. Such a relationship has certainly been indi-

cated for human mosquito-borne filariasis (11, 12). It would follow that vector control, if imperfect and unsupported by other measures, could increase the microfilaraemia rate (and microfilarial densities),

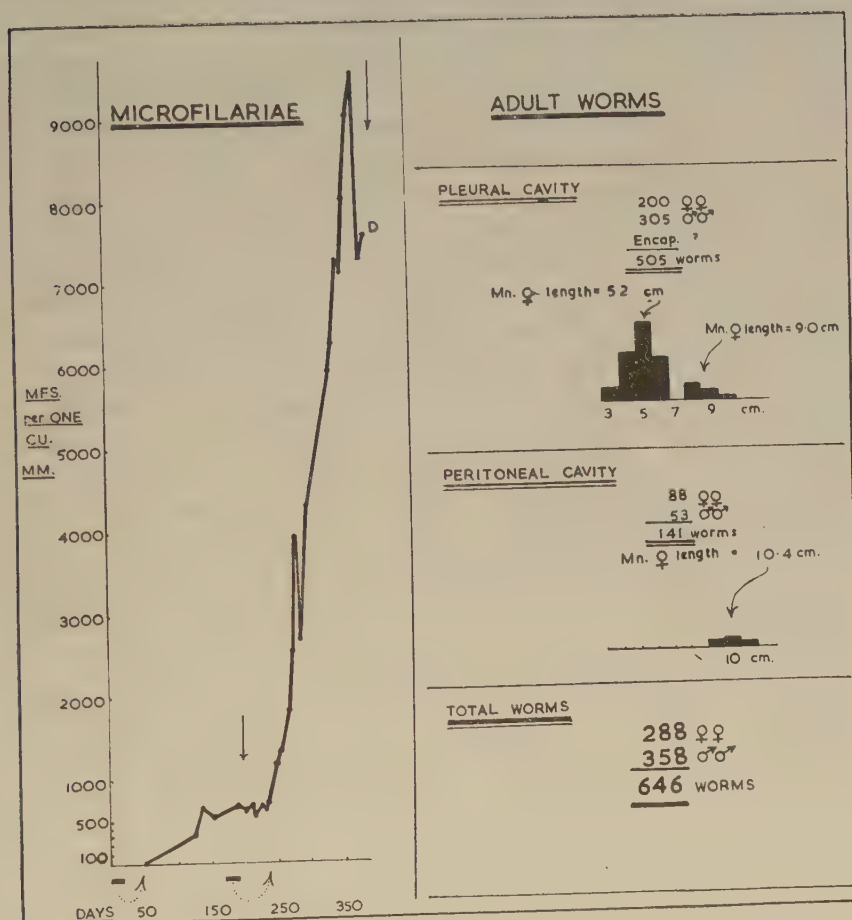


Fig. 2 — Illustrating a very heavy microfilaraemia following intermittent transmission. The peritoneal worms probably contributed substantially to the high microfilarial counts resulting from the second transmission period

although ameliorating disease manifestations. This may have happened in one control scheme in human filariasis (13). If, on the other hand, filarial disease is caused by microfilariae, the risk of developing and

intensifying the condition seems greatest with intermittent transmission, whether natural (seasonal) or the result of imperfect vector control.

Are the host-parasite relationships in cotton rat filariasis unique to this infection? It is well known that different filarioid species parasitize specific tissue systems in the host. It is probable that each species manifests infection, whether in the form of filarial disease or as a microfilaraemia, at quantitative levels of parasitization peculiar to the species in its specific tissue environment in the host. Combination of these characteristics—parasitological and numerical—may be obscuring that, fundamentally, host-parasite relationships of the same nature—and potentially with the same consequences—as those observed in cotton rat filariasis are at play in other filarioid infections. In cotton rat filariasis selective distribution of developing and adult worms between different sites within the parasitized tissue system is a fundamental fact which closely affects the expression of the infection. It may be emphasized that the fate in the mammal of the majority of infective larvae of human, and the related, filarioids is not well understood. Only a few specimens are subsequently found as developing or adult worms, or their original existence may be proven only by the presence of microfilariae. There seems good reason for investigating the early development of filarioids in general in their vertebrate hosts, perhaps by marker technique (14), to elucidate their host-parasite relationships in terms of (a) primary and secondary (alternative) sites of development, (b) effects of this on worm development and fecundity, (c) effects of frequency and amount of reinfection on worm distribution and vigour, and (d) the consequences in microfilaraemia and in filarial disease of the interactions of these factors.

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POSSIBLE STEPS IN THE EVOLUTION OF FILARIAL LIFE CYCLES

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INTRODUCTION

Although the first filarial life cycle was elucidated by Sir Patrick Manson over three-quarters of a century ago, there has not been a satisfactory hypothesis to explain the origin of these remarkable life cycles. Their outstanding feature is the fact that the early larvae have no route of exit from the body of the definitive host except by the intermediary of a haematophagous intermediate host. The central problem is to explain how these nematodes came to establish themselves in the host's tissues and how they became associated with these arthropods. The lack of knowledge of the life cycles of all but the most specialized filariae, such as those in man, has hitherto prevented helminthologists from envisioning possible steps in this evolution. Recent findings in general nematology make it possible, however, to suggest possible steps and to make a preliminary contribution to this problem, that is not without some interest and significance to tropical medicine.

GENERAL CONSIDERATIONS

In the following discussion we rely on the hypothesis that the intermediate host was acquired some time after nematodes became parasites of vertebrates (Chabaud, 1955). It is assumed, furthermore, that the filarioids and the spirurioids had a common ancestor that

was probably not unlike certain members of the family *Thelaziidae* (Chitwood and Wehr, 1934). Chabaud (1955a) has concluded, after a study of the cephalic structures found in filarioids and spiruroids, that a thelaziid-like ancestor gave rise to two lines of evolution, one leading to the filarioids, the other to the spiruroids.

POSSIBLE STEPS IN THE EVOLUTION

Presumably the ancestors of both the spiruroids and filarioids were parasites of the intestines of their hosts. They probably had life cycles like those of the primitive spiruroids, in which the eggs pass out in the faeces and are ingested by coprozoic intermediate hosts, the latter being ingested finally by the definitive host, e.g., *Rhabdochona ovifilamenta* of the *Thelaziinae* (Weller, 1938). Presumably these early parasites were able to use a great many intermediate hosts, there being little specificity at this stage of their evolution (Chabaud, 1955).

I. *The adults establish themselves in the orbit.*

The first step envisioned in the evolution of filarial worms from such an ancestor was for the worms to establish themselves in the orbital cavities. Their eggs still passed out in the faeces and were ingested by coprozoic intermediate hosts. Example: *Oxyspirura mansoni* (*Thelaziinae*). This species, living in the orbits of fowl, produces thick-shelled eggs that pass through the lacrymal duct and out in the faeces. The eggs hatch and the larvae develop in *Pycnoscelus*. When infected sow-bugs are eaten, the liberated, infective larvae migrate to the orbit, where they reach adulthood (Fielding, 1927).

II. *The larvae accumulate in the orbit.*

The larvae remained in the orbit from which they were ingested by various arthropods that fed there. Some of these arthropods became intermediate hosts. The infective larvae left the intermediate host as it was feeding and entered the orbit, where they reached adulthood. One can visualize a life cycle in which coprozoic arthro-

pods and arthropods that fed about the orbit were involved in the life cycle at the same time. Example: *Thelazia rhodesii* (*Thelaziinae*). This species lives in the orbits of cattle and their larvae stretch the egg capsule and accumulate in the lachrymal secretions. Muscoid flies ingest the larvae and serve as intermediate hosts. The infective larvae leave the feeding flies and crawl into the orbit, where they reach adulthood (Krastin, 1959).

III. *The adults enter the subcutaneous tissues.*

The adults established themselves in the subcutaneous tissues of the head and neck. They returned to the orbit and oviposited their larvae, which, as in *Thelazia*, were ingested by intermediate hosts that fed there. Example: There is no proof that such a life cycle exists, but there is circumstantial evidence that it may. *Souamofilaria coronata* of *Coracias* spp. is generally reported from the subcutaneous tissues of the head and neck. This species, however, has been reported at least once from the orbit (Boulenger, 1928) and one species *Squamofilaria sicki* is known only from the orbit. We have suggested, on the basis of these observations, that these worms, although living in the subcutaneous tissues of head and neck, may return to the orbit and oviposit their eggs (Anderson and Chabaud, 1958). Conceivably, they have life cycles like *Oxyspirura* spp. Perhaps life cycles like those of *Thelazia* will be found among the filariids (*Filariidae*) that live under the skin of their hosts. This step was not postulated by Anderson (1957).

IV. *The adults become independent of the orbit.*

Instead of returning to the orbit, the female pierced the skin of the definitive host and deposited her eggs to the outside in a bleeding lesion. The lesion attracted haematophagous arthropods that ingested the eggs and larvae. Some of these arthropods would probably have been the same intermediate hosts that originally took the larvae or eggs from the orbit, e.g., *Musca* spp., but possibly other intermediate hosts were acquired at this time. The infective larvae left the intermediate host as the latter fed about various sores and scratches on the host's body and penetrated into the subcutaneous tissues where

they reached adulthood. Example: *Parafilaria multipapillosa*. The adults live under the skin of horses. The female worm makes a minute hole in the skin which bleeds profusely and into which she protrudes her head and deposits her eggs. Muscoid flies swarm onto the lesion and certain species, after ingesting the eggs, serve as intermediate hosts (Baumann, 1948). The route of infection of the definitive host is still unknown, but we suspect that it is through sores and abrasions on the body of the host.

V. *The larvae accumulate in the tissues about the female.*

The larvae invaded and accumulated in the tissues about the female worm. The presence of the worms elicited an ulcerated skin lesion in which the larvae accumulated. This sore attracted arthropods that served as intermediate hosts. The infective larvae left these intermediate hosts, as the latter fed about lesions of various kinds on the body of the host, and penetrated into the subcutaneous tissues, where they reached adulthood. Example: *Stephanofilaria* spp. The life cycles of these worms are still unknown, but they are always associated with localized skin lesions in which are found the larvae and the adults. Buckley (1937) and Dikmans (1934) have reported that various insects are attracted to these lesions. Undoubtedly some of these insects will be found to be intermediate hosts. Assuming that the microfilaria is basically a larva specialized to live in the tissues of the definitive host, it is conceivable that the microfilaria had its origin at this stage in the evolution of filarial worms.

VI. *The larvae accumulate in the subepidermis and skin-penetrating arthropods become involved in the life cycle.*

The larvae invaded the subepidermis and the dermis, where they became available to haematophagous arthropods able to pierce the skin. The latter became favoured intermediate hosts and they replaced those species unable to pierce the skin. Skin lesions associated with the larvae and the adults became unnecessary to the life cycle. The infective larvae entered the wound made by the feeding arthropod. Example: *Onchocerca* spp. The details of these life cycles are well known. The larvae of two species of *Dipetalonema* (*grassii* and *strep-*

toerca) accumulate in the skin of their hosts, although those of other members of the genus are found in the blood.

Anderson (1957) has supposed that skin lesion of the type found in *Stephanofilaria* attracted arthropods that could pierce the skin (mosquitoes, simuliids, etc.). There is no reason to make such a dubious assumption because one can postulate that the larvae radiated from lesions into healthy tissue from which they were ingested by arthropods that pierce the skin to obtain their food.

One would expect vectors like mosquitoes and simuliids to replace forms like muscoid flies. The former are probably more efficient vectors than the latter because they produce lesions which allow infective larvae to enter the host. The life cycles of species using muscoid flies are presumably dependent upon the vector being attracted to breaks in the host's skin. In addition, mosquitoes, etc., besides being specialized blood-feeders, are, unlike muscoid flies, often remarkably selective in their choice of animals on which to feed. Presumably, relatively few infective larvae are lost from such vectors. This is perhaps why most filarioids are transmitted by arthropods that can pierce the skin and groups associated with prominent skin lesions are small and taxonomically rather isolated (*Filariinae*, *Stephanofilariinae*).

VII. *The larvae accumulate in the blood.*

The larvae of certain species, using blood-sucking arthropods as intermediate hosts, entered the blood stream and were available to the vector, no matter where the adults lived in the definitive host. Example: *Wuchereria* spp. The details of these life cycles are well known. Conceivably the filarioids radiated to many of the deeper tissues and tissue spaces of the host primarily after this stage was passed in their evolution. The only skin lesions associated with the transmission is that made by the vector when it feeds (Gordon and Crewe, 1953).

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FILARIASIS IN THE AMERICAS

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The current status of human filarial infections in most of the Americas is poorly known. In some areas, the most recent data seem to be 30 years old; in others, such as Brazil, systematic surveys to determine the scope of the problem are still underway. As in many other regions of the world, filariasis has been masked by other more important diseases which now can be cured or prevented.

Wuchereria bancrofti, apparently introduced into the Americas, has managed to maintain itself in several areas in Central and South America and islands of the Caribbean. In general, filariasis is lightly regarded as a public health problem. Communities with microfilarial prevalence rates exceeding 15 percent have been reported only from British Guiana, St. Croix, and St. Kitts. Elephantiasis rates exceeding 5 percent of the population examined have been found in these three areas and Ponta Grossa, Brazil. The primary agent, responsible for lymphangitis, hydrocoele, and elephantiasis, is still being debated.

No animal reservoirs of *W. bancrofti* have been discovered. Transmission is primarily by *Culex fatigans* (= *quinquefasciatus*). Locally, species of *Anopheles*, especially *A. darlingi* in South America, are important secondary vectors.

Vector control, so successful in curbing malaria, has been less effective against *Culex fatigans*. Residual insecticides, when augmented by larviciding, kill reduce the *C. fatigans* population and infection rate in mosquitoes. Evidence from British Guiana and from Puerto Rico

indicates that introduction of modern sewerage will reduce both the vector population and the infection in the human population.

Diethylcarbamazine has been tested in experimental studies in St. Croix, Puerto Rico, British Guiana, Venezuela, Brazil. Although large scale use of this drug is underway in some endemic areas of Brazil, the results are not available to me. Excluding this latter study, the practical value of chemotherapy as a preventive or public health measure has not been determined.

AS FILARIASES EM DIU

ANANTA CAMOTIM VAGA

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Antes de entrar no assunto cumpre-me esclarecer que o principal motivo que me levou a apresentar este modesto trabalho, feito sem auxilio do laboratório e de outros meios adequados e indispensáveis para a organização dum trabalho digno de ser presente a qualquer congresso, foi simplesmente levar ao conhecimento dos Ex.^{mos} Congressistas a forma assustadora e silenciosa com que este flagelo está a propagar-se, com a ameaça de se generalizar a todo o mundo, e a necessidade urgente dum combate bem organizado com as normas internacionais, a fim de se conseguir um resultado satisfatório, embora não tenhamos na mão armas seguras; portanto, espero me desculpem quaisquer faltas e deficiências.

Vim a este distrito de Diu, nomeado delegado de saúde, em 1954, mas, antes de chegar a esta cidade, tive ocasião de examinar um doente, natural de Diu e que trabalhou na cidade de Goa nos últimos dez meses, com elefantíase do membro inferior, o qual me informou haver muitos casos da mesma doença em Diu. De modo que já vim ciente e preparado para iniciar um possível estudo e um combate contra a mesma doença, trazendo comigo corantes, solução de D. D. T., etc.

Fiquei surpreendido quando soube que a maioria dos doentes eram tratados como cardíacos, renais, etc., e que a verdadeira causa da sua doença era quase desconhecida. Não me parece novidade este facto estranho, em virtude de, até essa data, se admitir a existência de filariase (elefantíase) somente em certas regiões da União Indiana, Paquistão, Arábia, África, etc., conhecidas como endêmicas e poucos terem pensado na possibilidade da entrada desta doença em territórios onde antes nunca existira.

O mais antigo caso que encontrei teve início há quase 50 anos, passando despercebido durante todo esse período, o mesmo sucedendo na cidade de Goa, onde, apesar de contar com tantos clínicos distintos e de existirem alguns gabinetes de análises, hospitais, a Escola Médico-Cirúrgica, etc., somente após 25 anos, aproximadamente, foi aceite a verdadeira origem do flagelo e iniciada a campanha contra o mesmo.

Este facto demonstra claramente o verdadeiro perigo desta doença ao passar despercebida durante longos anos e que o diagnóstico só é aceite quando uma grande percentagem da população se encontra infestada, com a manifestação de microfilárias no sangue periférico e um bom número de casos de elefantíase. Por isso, sou de parecer que, em face de todo o edema localizado e de sinais objectivos de filariase que não justifiquem outra causa, não se deve eliminar a hipótese de filariase pelo facto de antes nunca se ter registado um só caso na localidade, a fim de se evitar a sua propagação, não só em Diu e Goa, como também em certas outras regiões, onde, salvo opinião em contrário, somente foi diagnosticada e aceite a sua verdadeira origem após o mal ter alastrado bastante.

E, como o portador de microfilárias, ao deslocar-se a qualquer parte do mundo onde exista o mosquito transmissor, pode ser o veículo de propagação da doença, é desde já conveniente pensar em tomar as medidas internacionais necessárias para se evitar que a mesma se expanda.

A cidade de Diu está situada na ilha do mesmo nome, no golfo de Cambaia, à latitude de 20° N.; tem um clima bastante variável, sofrendo constantes alterações de temperatura, que oscila entre 39° e 18° na época quente e entre 31° e 7,5° na época fria, e fraca pluviosidade. Sabida a sua localização na costa da península de Catiavar, julgo desnecessária uma descrição pormenorizada das condições climatéricas, que são bastante conhecidas: média térmica anual, cerca de 26°C; humidade relativa, 75,3.

A cidade de Diu é pequena, com muitas casas em ruínas, alguns poços e cisternas abandonados; por falta de esgoto apropriado, as águas domésticas são recolhidas em reservatórios a que os naturais dão o nome de *cundi*, onde as mesmas estagnam, dando origem ao mosquito *Culex*. enquanto poços abandonados, tanques, charcos e

cisternas contribuem para a criação do *Anopheles*. Cidade aonde, devido ao sezonismo, nenhum indivíduo doutros distritos se atrevia a vir, com a verificação da existência de filariase foi colocada numa situação assustadora quanto ao estado sanitário. A sua população actual é aproximadamente de 4000 habitantes, mas outrora devia ter sido muito elevada, atingindo mais de 100 000, o que não repugna admitir em face das ruínas presentemente existentes. Mas a emigração da população para as províncias da África e o intenso sezonismo reduziram consideravelmente a população.

GRAU DE INFESTAÇÃO DA POPULAÇÃO
POR *WUCHERERIA BANCROFTI*

A fim de determinar o grau de infestação da população, colheu-se sangue de 1995 indivíduos, indiferentemente sãos, com sinais objectivos e com elefantíase.

Entre estes, 201 tinham microfilárias *Bancrofti* no sangue periférico, o que equivale a 10 por cento dos infestados.

$$\text{Número total das colheitas — 1955} \left\{ \begin{array}{l} M = 951 \\ F = 1044 \end{array} \right.$$

$$\text{Número total das positivas — 201} \left\{ \begin{array}{l} M = 93 \\ F = 108 \end{array} \right.$$

QUADRO 1 — *Idades dos individuos cujo sangue foi analisado e dos portadores de microfilárias, com as respectivas percentagens*

Designação	Até 5 anos	6 a 10 anos	11 a 20 anos	21 a 30 anos	31 a 40 anos	41 a 50 anos	51 a 60 anos	Mais de 60 anos
Número total . .	43	187	485	644	230	212	126	68
Número de porta- dores	2	7	48	80	20	24	16	4
Percentagem . . .	4,6	3,7	9,8	1,24	8,6	11,3	12,6	5,9

Entre os indivíduos com sinais objectivos de filariase, incluindo quistos sinoviais, 6 foram positivos e com elefantíase e nenhum apresentou microfilárias no sangue periférico.

Em relação ao sexo, não houve diferença sensível, sendo a percentagem de 10, tanto nos homens como nas mulheres.

Em relação à idade, os indivíduos entre 21 e 30 e entre 41 e 60 anos apresentaram uma percentagem sensivelmente alta, enquanto nos com idade inferior a 10 anos e superior a 60 a percentagem foi bastante fraca.

Em 3 casos com infestação intensiva por microfilárias (mais de 150 por 20 mm^3) foi recolhido o sangue durante o dia (ao meio-dia, às 16 e às 19 horas), tendo-se verificado resultado positivo em todos os casos, mas em 2 casos com percentagem fraca (menos de 4 microfilárias por 20 mm^3) o resultado foi negativo no sangue periférico recolhido às mesmas horas.

Nestes indivíduos infestados de microfilárias, entre outros sintomas alérgicos já conhecidos, tais como urticária, febre, bronquites asmátiformes, cefaleias, etc., observei 2 síndromas importantes que melhoraram com Hetrazan; por isso, como casos que merecem especial atenção para se apurar se, de facto, a presença de microfilárias em abundância no sangue pode ser responsável, só por si, pelos mesmos síndromas, segue a descrição dos mesmos:

1.º *Síndrome com hemoptises.*

a) Mulher, 30 anos de idade, casada, estado geral bom, antecedentes pessoais e hereditários sem importância: vem sofrendo de hemoptises com tosse e temperatura ligeira há 2 anos. Segundo as informações prestadas pela doente, é tratada, após prévio exame radiológico no estrangeiro, com estreptomicina e outros medicamentos anti-tuberculosos. Quando tive ocasião de a observar, fui do mesmo parecer, embora à auscultação não se notassem quaisquer sinais anormais, baseando-me no diagnóstico anterior e nos sintomas descritos e continuando o mesmo tratamento, ainda que não tivesse observado melhoras. E um dia, quando foi analisado o sangue periférico durante a noite (na prospecção de porta-germes no mesmo bairro), verificou-se ser fortemente positivo quanto a microfilárias, com mais de 200 micro-

filárias por 20 mm³ de sangue (aproximadamente). Foi instituído o tratamento por Hetrazan, que, no início, provocou certas reacções alérgicas, como cefaleias, vômitos e ligeiro aumento de temperatura. Completo o tratamento de 3 semanas, com 6 comprimidos de Hetrazan por dia, não se repetiram as hemoptises nem outras perturbações e a doente encontra-se boa.

b) Mulher, 45 anos de idade, casada, estado geral bom, sem nenhuma anormalidade. Apresenta-se na consulta por ter hemoptises ligeiras, 4 a 5 vezes ao dia, e tosse seca asmatiforme. Na auscultação nada de especial, a não ser um ou outro ronco e sibilo. Temperatura normal. Interrogada, informa serem as hemoptises diurnas, passando bem durante a noite. A análise do sangue mostra microfilárias, aproximadamente 200 por 20 mm³. Para se apurar se as hemoptises tinham alguma relação com a periodicidade das microfilárias, embora a doente já tivesse respondido acima serem elas diurnas, solicitou-se a observação rigorosa durante 3 dias. Passado este período, a doente confirma serem as hemoptises diurnas. Tratada com Hetrazan durante 3 semanas, encontra-se livre do mal.

2.º *Síndrome mental.*

a) Mulher, 22 anos de idade, casada; teve o primeiro parto há um ano e nessa altura adoeceu com o síndrome mental depressivo, com apatia completa pelo trabalho e pelo falar, preferindo estar só, sem se importar com coisa alguma à sua volta, e desinteresse pela comida. Dorme pouco. Interrogada, não responde a nenhuma pergunta. Reflexos rotulianos ligeiramente aumentados; nenhum outro sinal de importância. Sujeitou-se durante um ano a variados tratamentos sem eficácia. O exame da urina revela quilúria. A análise do sangue acusa a presença de microfilárias abundantes. Tratamento por Hetrazan durante 3 semanas. Cura clínica da doente, desaparecendo as microfilárias do sangue e a quilúria e estando apta para todos os trabalhos.

b) Homem, 60 anos de idade, pedreiro, estando há mais de 4 anos em casa, considerado como louco, com sintomatologia depressiva, idêntica à do caso anterior, mas menos acentuada; a análise do sangue acusou microfilárias abundantes. O tratamento por Hetrazan provocou ligeiras melhoras.

c) Rapaz, 16 anos de idade, há muito tempo com sintomas depressivos, com tendência para a sonolência e diminuição das faculdades mentais ou, melhor, imbecilidade. Análise do sangue fortemente positiva quanto a microfilárias (134 por 20 mm^3 aproximadamente). O tratamento com Hetrazan produziu melhoras sensíveis.

d) Mulher, 35 anos de idade, casada, estado geral bom; queixa-se de insónias, apatia pelo trabalho e pela fala, durando a fase 7 a 8 dias. Apresentou semelhantes crises duas vezes nos últimos 2 anos, sendo esta, a terceira, observada por mim. A análise do sangue acusa microfilárias, mas raras (8 por 20 mm^3). Tratamento por Hetrazan. A doente encontrava-se melhor mesmo antes de iniciar o referido tratamento.

e) Homem, 35 anos de idade, solteiro, estado geral bom. Sintomas de imbecilidade há muitos anos (mais de 12), sendo incapaz de exercer devidamente qualquer profissão. Apresenta microfilárias no sangue. O tratamento não foi seguido rigorosamente. O doente continua no mesmo estado, já habituado a este modo de vida há muito tempo.

Devido à falta dos meios adequados, não foi possível efectuar as análises e exames necessários dos doentes acima referidos, mas julgo que não será de desprezar a minha conclusão, baseada na eficácia do tratamento, na possibilidade de certos síndromas mentais e hemoptísicos, manifestados pela presença de microfilárias *bancrofti* no sangue, ao lado de outros já sobejamente conhecidos, pedindo a atenção dos colegas para a verificação da presença de microfilárias, nestes síndromas, nas zonas endémicas, a fim de se poder confirmar esta conclusão.

Em 18 casos que tinham microfilárias abundantes, tendo-se recolhido o sangue após o tratamento rigoroso de 6 dias com Hetrazan, verificou-se que 11 eram negativos e 7 tinham poucas microfilárias (1 a 4). E após 21 dias de tratamento (2 mg por quilograma de peso 3 vezes ao dia) todos foram negativos, à excepção de um único, que ainda apresentava 1 microfilária. Entre os portadores de microfilárias tratados devidamente somente 3 apresentaram edemas, sendo um no braço e outros dois na perna, passados 4 meses; foi um destes que continuou a ter microfilárias após o tratamento completo de 21 dias.

Microfilarias perstans foram verificadas nos soldados africanos de Angola. Entre 175 soldados, 18 foram positivos quanto a microfilá-

rias *perstans* e 8 apresentavam tanto *perstans* como *bancrofti*. Para estudar um elemento da sua biologia recolhi sangue de 6 em 6 horas, durante dia e noite, e verifiquei que todas as placas eram positivas, sem distinção da hora de recolha do sangue, o que, ao lado da sua forma muito estreita, espirais múltiplas, movimentos rápidos (observados na placa fresca coberta por uma lamela), o número quase incontável, demonstra que as mesmas não obedecem a periodicidade como outras.

Tratei os mesmos doentes com Hetrazan, que, após 3 dias, fez desaparecer as microfilárias do sangue, mas, logo depois do início do tratamento, apresentaram acentuadas manifestações alérgicas, como urticária generalizada, febre, vômitos e edemas dolorosos em diversas partes do corpo, que desapareceram com a sua interrupção.

SINAIS OBJECTIVOS DE FILARIÁSE

Os casos com sinais objectivos de filariase foram relativamente poucos, sendo o seu número quase metade do dos casos de elefantíase. Entre 2000 pessoas observadas para pesquisa de sinais objectivos de filariase e elefantíase, foram os seguintes os números dos casos com sinais objectivos: com orquite, 10; com hidrocele, 8; com adenites inguinais, 5; com hipertrofia do gânglio epitrocliano, 3; com linfadenoma, 2; com quistos sinoviais ¹, 8.

$$\text{Número total} - 36 \left\{ \begin{array}{l} M = 24 \\ F = 12 \end{array} \right. \quad \text{Porcentagem: } 1,5.$$

A percentagem nos homens é quase dupla da das mulheres.

¹ Os sinais descritos já se acham sobejamente conhecidos, com excepção dos quistos sinoviais. Não conheço ninguém que tenha atribuído à filariase a origem dos mesmos quistos ou de quistos de outra espécie, mas foi em virtude de os 5 doentes com quistos terem sido positivos quanto a microfilárias e da sua frequência na zona endémica de filariase que me atrevi a incluir estes sinais neste capítulo, pedindo aos colegas a verificação do mesmo facto quando tenham oportunidade, para sua confirmação.

Entre estes 36 doentes, sômente 6 apresentaram microfilárias, e 5 destes tinham quistos sinoviais.

Os casos de hipertrofia do gânglio epitrocliano foram unilaterais, com edema da região correspondente, desaparecendo após o tratamento com Hetrazan. Todos os casos de quistos sinoviais foram observados nas mulheres.

QUADRO 2 — *Frequência dos casos com sinais objectivos de filariase, conforme o sexo e idades*

Designação	Crianças	Adolescentes	Adultos	Velhos	M	F	Total
Orquite.	—	2	4	4	10	—	10
Hidrocele.	1	4	2	1	8	—	8
Adenites inguinais . .	2	3	—	—	2	3	5
Hipertrofia do gânglio epitrocliano	—	2	1	—	2	1	3
Quistos sinoviais. . .	1	2	3	2	—	8	8
Linfadenoma	—	—	2	—	2	—	2

Quanto ao tratamento destes casos, farei a referência no capítulo apropriado.

ELEFANTÍASE

A característica dos edemas foi a seguinte:

A princípio o edema é fugaz e localizado no pé ou na perna (mais frequente), em seguida, na mão ou no antebraço. Este edema aparece, as mais das vezes, sem nenhum sintoma geral, como febre, dor, etc., e passados alguns dias desaparece espontâneamente, facto que leva o doente a desprezã-lo, attribuindo-o simplesmente a qualquer mudança de tempo. Mas, passados poucos meses ou anos, aparece de novo e torna-se estável, e é nessa altura que o doente pensa recorrer ao médico. Raras vezes esses edemas são precedidos de febre, vômi-

tos, cefaleias, linfangites, com características de centrífugas, aparecendo o edema clássico no fim. Os mesmos edemas não apresentam outras características especiais; alguns são duros, outros moles; uns deixam impressão digital, outros não, aparecendo a dureza conforme a antiguidade.

As localizações mais frequentes são no membro inferior e, em seguida, no membro superior.

QUADRO 3 — *Casos de elefantíase*]

Localização	Até 10 anos		11 a 20 anos		21 a 30 anos		31 a 40 anos		41 a 50 anos		51 a 60 anos		Mais de 60 anos	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Membro superior.	—	—	1	2	1	—	1	1	1	2	—	—	—	—
Membro inferior	—	1	3	1	—	12	1	14	5	5	1	2	2	1
Escroto	—	—	1	—	—	—	—	—	—	—	—	—	—	—
Escroto e membro inferior. .	—	—	—	—	—	—	—	—	—	—	1	—	1	—
Escroto e pênis.	—	—	—	—	—	—	1	—	—	—	—	—	—	—
Total	—	1	5	3	1	12	3	15	6	7	2	2	3	1

Verifica-se pelo quadro 3 que o número de casos de elefantíase nas mulheres é mais do dobro do dos homens ($M = 20$; $F = 41$), enquanto o dos portadores de microfilárias é sensivelmente igual e o dos com sinais objectivos é inverso, isto é, o dobro nos homens.

A percentagem dos casos de elefantíase do membro inferior foi de 77, vindo a seguir a dos do membro superior, enquanto outras localizações são raras. Não encontrei qualquer caso de elefantíase associada de ambos os membros, nem de seio ou outra.

Eis a descrição de 3 casos antigos, com as respectivas fotografias:

Caso n.º 1 — O mais antigo que encontrei. Como se vê, é elefantíase do membro inferior direito, atingindo volume razoável, cujo começo data de há quase 50 anos. O doente informou ter começado

com adenite inguinal do mesmo lado, que supurou e foi atribuída a peste bubônica, que ao tempo grassava na região (fig. 1).



Fig. 1

Caso n.º 2 — Elefantíase do escroto e membro inferior, com início há mais de 20 anos. Vê-se que o pênis desapareceu na massa escrotal, saindo a urina pelo orifício encontrado na parte superior do escroto (fig. 2).



Fig. 2

Caso n.º 3 — Elefantíase do membro inferior esquerdo, com início há mais de 20 anos (fig. 3).



Fig. 3

São os casos mais avançados e mais antigos de elefantíase observados em Diu. Entre outros casos de elefantíase do membro inferior somente 8 são de volume sensivelmente igual ao da fig. 3, sendo outros de volume bastante reduzido.

Elefantíase do pênis e escroto foi o único caso que observei após a operação a que o doente se sujeitou no estrangeiro (Bombaim).

O quadro 4 dá a ideia precisa do desenvolvimento dos casos de elefantíase segundo o período decorrido desde o começo da doença, sendo a sua propagação quase nula nos primeiros 30 anos, em que só se registaram 2 casos, depois foi aumentando, atingindo o máximo após 40 anos, verificando-se uma ligeira melhora a partir do começo da campanha, isto é, durante os últimos 4 anos. Eis o mapa:

QUADRO 4 — *Desenvolvimento dos casos de elefantíase segundo o período decorrido desde o começo da doença*

	Há quase 50 anos	21 a 40 anos	10 a 20 anos	5 a 9 anos	2 a 4 anos	Menos de 2 anos
Número de casos .	1	2	9	20	17	12

PROFILAXIA

Consistiu em:

1) Luta contra os mosquitos, de que, embora não tenham sido estudados, por falta de tempo e meios necessários, foi possível a verificação, que demonstrou a presença do *Culex* e do *Anopheles* (procedeu-se somente à classificação do género), desenvolvendo-se aqueles nas águas estagnadas de origem doméstica e estes nos poços, cisternas e tanques abandonados; procedeu-se à campanha antilarvar sistemática, aplicando-se semanalmente soluções fracas de D. D. T. nas coleções aquáticas responsáveis pela criação de larvas e fazendo-se periodicamente aplicações de solução a 5 por cento de D. D. T. nas paredes internas das casas dos bairros pobres e outros fortemente infestados.

2) Prospecção de porta-germes, procedendo-se ao tratamento compulsivo sistemático dos portadores de microfilárias, cujo número foi de 201. O tratamento consistiu na administração de Hetrazan, 2 mg por quilograma de peso 3 vezes ao dia, associado sempre a Antisan, a fim de se evitarem as reacções alérgicas. Nos casos de infestação intensiva por microfilárias iniciou-se o tratamento com doses muito pequenas, 25 mg a 50 mg de Hetrazan por dia, associado a 1 a 2 comprimidos de Antisan, o que contribuiu para evitar as reacções acen tuadas.

TRATAMENTOS DOS DOENTES COM ELEFANTÍASE E SINAIS OBJECTIVOS

Sujeitaram-se ao tratamento 48 doentes de elefantíase e 18 com sinais objectivos. O tratamento foi o seguinte: nos adultos, Hetrazan durante 3 semanas, 2 mg por quilograma de peso, 3 vezes ao dia,

associado a antiomaline, série de 10 injeções, com intervalo de 2 a 3 dias; nas crianças, metade da dose. Este último produto somente provocava dores fortes na região das injeções, motivo por que muitos doentes interrompiam o tratamento.

Entre os doentes de elefantíase 5 apresentaram cura clínica completa, de início recente; quase todos os outros melhoraram bastante com a redução muito sensível do edema. Nos casos antigos o tratamento somente contribuiu para se evitarem e aliviarem as complicações, tais como linfangite com febres, etc.

Certos edemas recentes e casos de orquite e adenites inguinais desapareceram com 3 a 4 injeções de antiomalina.

Entre os 18 casos com sinais objectivos somente os de orquite, hipertrofia do gânglio epitrocliano, adenites inguinais e linfadenomas melhoraram consideravelmente com o tratamento estabelecido, enquanto os de hidrocele e quistos sinoviais não reagiram ao tratamento.

RESUMO

Durante um inquérito sobre as filariases em Diu chegou-se às seguintes conclusões:

Nesta cidade a doença começou há 50 anos, passando despercebida a sua origem durante quase meio século.

O grau de infestação da população da cidade é de 10 por cento. A periodicidade das microfilárias foi nocturna, mas, conquanto a infestação fosse acentuada, encontraram-se microfilárias durante o dia, em pequeno número. A percentagem de infestados por microfilárias foi maior nos indivíduos aparentemente sãos, fraca nos com sinais objectivos e nula nos com elefantíase. A percentagem dos portadores de elefantíase é de 2,5.

O tratamento por antimoniais com Hetrazan mostrou-se eficaz nos casos recentes de elefantíase, reduzindo o edema e impedindo-o de atingir volumes enormes. O Hetrazan associado a Antisan, nos portadores de microfilárias, foi bem tolerado, sem reacções alarmantes, e mesmo em pequenas doses fez desaparecer microfilárias do sangue, em poucos dias, mas nem sempre evitou que certos doentes apresentassem sinais objectivos de elefantíase. Certas hemoptises e perturbações mentais são de atribuir à infestação acentuada de microfilárias, confirmadas pela cura com Hentrazan.

Em Diu a infestação é por *F. bancrofti*; foram encontradas microfilárias *pers-tans* nos africanos de Angola, mas em nenhum natural.

Perante qualquer edema dos membros ou outro sinal objectivo de filariase que não justifique outra causa, independentemente do exame laboratorial, que

geralmente é negativo nesses casos, não se deve excluir a hipótese de filíase pelo facto de anteriormente nunca se haver registado qualquer caso na localidade.

Seria, pois, de recomendar a exigência a todo o viajante internacional procedente das zonas infestadas de filíase de um cartão sanitário provando não ser portador de microfilárias, como medida de *contrôle* da doença, a fim de, desde já, evitar a sua propagação.

THE EXPERIMENTAL TRANSMISSION OF *WUCHERERIA* INFECTIONS FROM MAN TO ANIMALS

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Very few attempts to transmit *Wuchereria* experimentally from one vertebrate host to another appear to have been made. Yokagawa (1939) reported the successful infection of two human volunteers by the bite of mosquitoes infected with *W. bancrofti*. Microfilariae were seen in blood films 82 and 115 days after infection, but unfortunately the evidence is not conclusive, as the microfilariae were found in blood films on one occasion only and it is not certain whether the volunteers had been in an endemic area.

Hodgkin (1939) attempted to transmit *W. malayi* from human carriers in Malaya to the long-tailed macaque monkey (*Macaca irus*) by mosquito bite. The monkeys were still negative 6-12 months after being bitten. The finding of *malayi*-like microfilariae in a number of domestic and forest animals in Malaya (Edeson, Wharton and Buckley, 1955) led to further attempts to transmit *W. malayi* from man to a number of species of animals.

EXPERIMENTAL TRANSMISSION OF HUMAN STRAINS OF *W. MALAYI* TO ANIMALS

a) The first successful transmission of *W. malayi* from man was achieved in the domestic cat (Edeson and Wharton, 1957). Infective larvae were obtained from laboratory-bred *Mansonia uniformis* which

had fed on human carriers in Pahang, Malaya — the larvae were suspended in saline and injected subcutaneously into cats. Microfilariae appeared in the blood 80-96 days after inoculation. These experiments established that human strains of *W. malayi* could be transmitted to an animal and that the infections behaved apparently normally in the new host.

b) Later, other species of animals were infected with Pahang strains of *W. malayi* using the syringe inoculation technique. These were the long-tailed macaque and rhesus monkeys, the slow loris and the civet cat (Edeson and Wharton, 1958). The different species of animals varied in the ease with which they could be infected. The domestic cat was readily infected while the long-tailed macaque monkey was difficult. Attempts to infect white mice, a guinea pig and a pig-tailed macaque failed.

c) Two out of three cats have recently been infected by mosquito bites, thus confirming the part mosquitoes play in the life cycle of the parasite (Wharton, Edeson and Laing, 1958).

d) The course of the transmitted infection varied according to the species of animal infected. The pre-patent period in cats was short (76-98 days), microfilaria counts rose rapidly to relatively high levels (>100 per 60 mm^3 blood) and the microfilaraemia persisted for a year or more. The pre-patent period in both species of monkey was short (77-93 days), but while the microfilaraemia rose steadily in the rhesus monkey, in the long-tailed macaque the counts did not increase greatly. The pre-patent periods in the slow loris were usually prolonged (81-256 days) and in the only civet cat infected the pre-patent period was also prolonged (153 days); the microfilaraemia in these two species, although persisting for up to one year, was always at a low level (>50 per 60 mm^3 of blood). The microfilariae in the experimentally infected animals appeared to be identical to those in the human carriers as far as morphology and behaviour in vertebrate and insect host were concerned. The microfilariae developed to maturity in several species of *Mansonia* mosquito and the infective larvae produced were capable of developing in other animals of the same or a different species (Fig. 1).

e) Following the transmission of the Pahang strains of *W. malayi* attempts were made to transmit strains occurring in human carriers from Kedah, North Malaya. *W. malayi* in Kedah behaves differently

and life history of *W. malayi* in the vertebrate host and they throw some light on the conditions found in nature.

1. *The development of W. malayi in the vertebrate host.*

a) The pre-patent period, between inoculation of infective larvae and the first appearance of microfilariae, has consistently proved to be about 80 days in a suitable host (cat) with a minimum of 75 days so far recorded.

b) Infective larvae have been recovered from the lymphatic system of an experimentally infected cat within 16 hours of injection subcutaneously. Developing *Wuchereria* have been recovered from the vessels associated with most of the major groups of lymph glands, irrespective of the site of inoculation of the infective larvae.

c) Two moults have been observed in the development of the worm; one at 9 days and one at 40 days.

d) The longevity of the *Wuchereria* in the vertebrate host can now be assessed with some degree of accuracy following single inoculation of infective larvae.

e) The ability to recover reasonable numbers of *W. malayi* from experimental animals makes it possible to study the morphology of the adult worm in more detail and to determine the variations within the species. Although the microfilariae in carriers from Kedah differ so markedly in behaviour from those in carriers from Pahang, the morphology of the adults does not appear to differ sufficiently to justify creating another species.

f) It is now possible to study in detail the action of diethylcarbamazine on the microfilariae and adults of *W. malayi*. Preliminary observations suggest that to achieve complete destruction of the microfilariae much larger doses must be given to cats than have to be given to man. Death of the adult worms is achieved if large doses are given.

2. *The relation to conditions in nature.*

a) The ease with which the Pahang infection can be transmitted to cats in the laboratory is supported by the high infection rate found in cats in villages in Pahang; while the difficulty of infecting the long-

tailed macaque monkey, the slow loris and the civet cat is consistent with the low infection rate found in these animals in nature. Our information, however, is still scanty. The relative difficulty in transmitting Kedah strains is also supported by the low infection rate among cats in the Kedah villages.

b) The pre-patent period of 76-98 days for experimental infections in cats is in close agreement with the age of the youngest infant found infected in nature — $3\frac{1}{2}$ months.

c) Transmission of *W. malayi* in nature could be effected by *M. longipalpis*, which occurs in village and forest and which is very catholic in its biting habits. Precipitin tests have shown that in highly endemic filariasis areas this mosquito feeds on a variety of animals including buffalo/cattle (84 percent), man (6 percent), fowls (3 percent), goats (2 percent), cats (1 percent) and pigs (1 percent).

d) The presence of the closely related species *W. pahangi* (Buckley and Edeson, 1956) in animals is a complicating factor as the infective larvae of the two species are indistinguishable in the mosquito. Present evidence, however, suggests that *W. pahangi* does not occur in man. *W. malayi* has been found occurring naturally in man, the domestic cat, two species of monkey and the civet cat; while *W. pahangi* is known to occur in the domestic cat, dog, two species of wild cat, tiger, civet cat, moon-rat and slow loris. In addition, *malayi*-like microfilariae have been found in the pangolin, one species of tree-shrew and the giant squirrel.

e) Many *Mansonia* spp. caught in jungle swamp areas are found infected with larvae indistinguishable from those of *W. malayi*. Inoculation of these larvae into cats should provide a means of identification by examination of the adult worms when they have developed in the cats.

f) The potential animal reservoir in Pahang has not yet been fully examined, but, from the preliminary results of a mass treatment campaign with diethylcarbazine, the possibilities of humans being reinfected from animals appear to be considerable. In two small areas the microfilaria rates and the mean count per 20 mm³ of blood have shown a steady increase during a period of two years observation since treatment stopped and control had no apparent effect on mosquito transmission (Wharton *et al.*, 1958).

SUMMARY

Wuchereria malayi has been transmitted experimentally from man to animals by the direct inoculation of infective larvae.

Animals successfully infected were the domestic cat, long-tailed macaque and rhesus monkeys, the slow loris and the civet cat.

The domestic cat has also been infected by mosquito bite.

The animals varied in their suitability as host, the cat being the most suitable.

The microfilariae in the animals resembled those in the human donor in behaviour and morphology.

The microfilariae developed normally in the vector mosquitoes and the infective larvae developed in other animals.

The infections in cats lasted up to one year or more.

The possible applications of these experiments and the relations to conditions in nature are discussed.

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CHANGING CONCEPT OF *WUCHERERIA BANCROFTI* TRANSMISSION IN NETHERLANDS NEW GUINEA

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Filariasis in Netherlands New Guinea is caused by *W. bancrofti*. The microfilaria shows nocturnal periodicity. The statements in the literature about the existence in Melanesia of a non-periodic *W. bancrofti* rests rather on misunderstanding than on actual observation (Backhouse & Heydon, 1950). The presence of *W. malayi* has been confined to imported carriers; no sign of transmission originating from these imports has ever been found, in spite of a reasonable abundance of *Mansonioides* species in several localities.

The original suggestion expressed by Buxton (1927) about a possible role of anopheline mosquitoes in the transmission of *W. bancrofti* in Melanesia has been substantiated by Backhouse (1934), who observed full development in *A. farauti* and *A. punctulatus*, in the experiment. Especially in *A. farauti* an easy development into the infective larval stage could be demonstrated. Near Hollandia Toffaleti & King (1947) have incriminated all the members of the *punctulatus* group as natural vectors of *W. bancrofti*. Further investigations by several workers have supported the role of these anophelines and especially of *A. farauti* in Melanesia. Meanwhile the importance of anophelines in this transmission got further support by Elsbach (1937), who found *A. bancrofti* naturally infected in the south of New Guinea. All these data have brought Backhouse & Heydon (1950) to the conclusion that anopheline mosquitoes, and especially *A. farauti*, play the major role in the transmission of *W. bancrofti* in Melanesia. In

the experiment *A. karwari* proved to be hospitable (Metselaar, 1955). Moreover, natural infection in *punctulatus* group has frequently been observed in routine-dissections, carried out by the Division of Malaria, Hollandia, in search for sporozoites.

That anophelines play the major, if not only, role in the transmission of filariasis could in several areas be inferred from the epidemiological relationship between malaria and filariasis, the endemicity of both diseases being found repeatedly closely related.

Unexpectedly, however, each of us has found an area where we had to conclude that endemic malaria is absent, in spite of highly endemic filariasis. These areas are the Berau region in the west (De Rook, 1957a), and the Bamgi-Ia area in the south (Van Dijk, 1958).

Our latest investigations on the transmission of *W. bancrofti* in Netherlands New Guinea have pointed attention to the following mosquitoes:

I. *Culex fatigans* Wiedemann.

This recently imported mosquito has already multiplied into an appreciable population in many urban centres of New Guinea, from where, at some instances, a secondary invasion took place into the adjacent area. There are many contradictory data supplied by previous investigations on the hospitability of this species in the experiment in the western Pacific region. Different authors noticed complete development in a small proportion of this mosquito in New Guinea or surrounding islands (Brug & De Rook, 1953, and Backhouse & Heydon, 1950). In 1957 it was observed on the island Pam (Western New Guinea) that of 23 specimen 4 were naturally infected of which one showed mature larvae and 4 specimen fed on a microfilariae-carrier were all infected and showed normal larval development. It is only because of its relative scarcity that *C. fatigans* does not play a major role in the filariasis transmission on the island (De Rook, 1957b).

Although not the original vector of endemic filariasis in New Guinea, the comparatively recent increase in the numbers of *Culex fatigans*, especially in urban centres, does no longer allow to look upon it as a harmless species.

II. *Culex bitaeniorynchus* Giles.

This mosquito, known from Indonesia as a very poor biter of human being (Bonne-Wepster, 1956), has been encountered as a dominant species in the Berau region, very active, especially at night. In the experiment an infection rate of 87 percent has been obtained; the natural rate of infection with infective larvae being 2.1 percent proves it to be an efficient vector of *W. bancrofti* (De Rook, 1957a).

III. *Culex annulirostris* Skuse.

In the same area, *C. annulirostris* has been found in almost equal density, with similar figures for the infection rates: experimental infection 90 percent natural infection with infective stage larvae 1.6 percent (De Rook, 1957a). On the island of Pam a distinctly lower experimental rate has been found, although the natural infection rate with infective larvae was 2.3 percent (De Rook, 1957b).

IV. *Culex aquamosus* Taylor.

In one single specimen of this species used in the experiments complete development into the infective larval stage has been observed. Although this species is wide-spread in New Guinea, it never occurs in great numbers, its role as a vector of *W. bancrofti* therefore being doubtful (Van Dijk, 1958).

V. *Aedes kochi* Donitz.

Backhouse & Heydon (1950) have obtained full development in this species. This could be confirmed on Pam island, where an experimental rate of 70-75 percent has been reached. The high mortality in the experiments proved largely to be due to the experimental circumstances, as a natural rate of infection with infective larvae of 1.6 percent has been found (De Rook, 1957b).

VI. *Mansonioides uniformis* Théobald.

In the Berau region an experimental infection rate was found in this species of 90 percent. The natural rate of infection with full grown larvae was 1.3 percent (De Rook, 1957a). In the Bamgi-Ia area where *Mansonioides* species are highly prevalent, an experimental rate of 91 percent has been obtained (Van Dijk, 1958). The development into the infective larval stage was easy.

VII. *Mansonioides papuensis* Taylor.

This species can readily be infected in the experimental. Van Dijk (1958) obtained an experimental infection-rate of 96 percent, but the mortality under experimental conditions was very high. Only one specimen lived 12 1/2 days and this mosquito showed mature larvae. In spite of the high experimental infection rate, it is felt that more evidence is required about the actual hospitality of *M. papuensis*.

By all these data about the hospitality and natural infection of non-anopheline mosquitoes to *W. bancrofti* in New Guinea, the question has been raised to what extent these mosquitoes actually compete with the anophelines. To answer this question we have to take into consideration, besides the degree of hospitality of the species, their spread, abundance, longevity, biting habits and the distance of breeding places to the houses, together, of course, with the existence of a reservoir, which, as far as we know, is limited to the human population.

As our experiments have been carried out with mosquitoes spontaneously attacking in the evening and night, an adequate impression has been gained of the comparative ability of the various mosquitoes to ingest microfilariae in a village. The hospitality and the longevity have been established by the natural rate of infection with full grown larvae. We are aware of the fact that all these factors may differ locally and that seasonal influences may complicate this matter.

The 5 non-anopheline species of mosquitoes (*C. fatigans*, *C. bitaeniorynchus*, *C. annulirostris*, *Aedes kochi* and *Massonoides uniformis*) which were found naturally infected with mature larvae entered the houses for feeding on men and could be captured indoors in the morning. They were breeding close to the houses.

As far as our patchy knowledge permits some general statements, we feel justified in drawing the following picture:

The spread of anophelines is far beyond the limits of the filariasis endemicity. Even in areas where hyperendemic malaria has been found, filariasis proved to be absent. This phenomenon may be explained by a very short life-span of the abundant *punctulatus* group mosquitoes under the local conditions, this assumption being firmly supported by the low sporozoite rates collected by Metselaar (1957). On the other hand, on the island of Pam, where the distance between breeding places and the houses was not greater than 400 m, *A. farauti* showed a natural rate of infection with full grown larvae as high as 6.6 percent.

Elsewhere the endemic filariasis has been built up with the help of non-anopheline mosquitoes beside anophelines. In this respect the ubiquitous *C. annulirostris* needs attention, as this species breeds in a wide variety of waters and is well adapted to man-made breeding places. Of growing importance is *C. fatigans*. To determine the relative importance of these mosquitoes, much work has to be done yet, and it is probable that the transmission pattern will prove to differ from locality to locality.

Beside this general pattern, the local situations determine which species is to be the vector according to the breeding facilities, which are offered to a particular mosquito species or a well-defined group of species:

1. So the deforested hills around Hollandia invited *A. karwari*, its actual role in filariasis transmission is however not known.

2. Extensive *Pandanus* growths offer breeding places to *Aedes kochi*. This efficient vector seems widespread, although we are not yet well informed about the areas with abundant *A. kochi* breeding, as on Pam island (De Rook, 1957b).

3. The extensive fresh-water swamps preferred by *Taeniorhynchus* species, among them *M. uniformis* being the most common, are of special interest, as these swamps comprise an appreciable part of New Guinea, and as in a number of them the remarkable absence of anopheles breeding has been observed. Elsewhere *A. farauti*, *A. kolienensis* or *A. bancrofti* have been found side by side with *Taeniorhynchus* species. The role of *M. papuensis* in these areas needs further support; *T. crassipes* has never been met in sufficient numbers to be of possible importance. It is thought that *C. squamosus* has possibly a relation to

this type of breeding place, although only a few specimens have been caught. The same might hold for *C. bitaeniorhynchus*, the very efficient vector in the Berau region, although this area has been the only locality so far where this species has been found.

These data may be sufficient to indicate that the anopheline mosquitoes do not stand alone in the transmission of filariasis in Netherlands New Guinea. Our knowledge so far points to *Mansonioides uniformis* and *Culex annulirostris* as the next candidates, while *C. fatigans* is threatening.

It will be clear that these facts influence the control of filariasis. The indoor spraying with residual insecticides, primary directed against malaria, would have eradicated anopheline-borne filariasis silently. The *M. uniformis*, which bites in some places rather often outside houses, and the DDT-resistant *C. fatigans* will require other measures.

SUMMARY

There has been sufficient evidence to incriminate the *punctulatus* group anophelines comprising *A. farauti*, *A. punctulatus* and *A. koliensis* as vectors of the nocturnal *W. bancrofti* filariasis in Melanesia. Of these anophelines *A. farauti* is probably the most important carrier. In New Guinea also *A. bancrofti* and possibly *A. karwari* play an additional role in the transmission.

The suggestion that anophelines play the major, if not the only, role in filariasis transmission in New Guinea has been found needing some revision, since in Netherlands New Guinea natural infection with full grown bancroftian larvae has been encountered in 5 non-anopheline mosquitoes, while experimental infection with full development of the larvae into the infective stage has been observed in two other species.

Taking into account the hospitality, the spread, the abundance, the longevity, biting habits and the distance between human dwellings and breeding sites of these non-anopheline vectors, the evidence is growing that some of these species really compete with the anophelines in efficiency to transmit *W. bancrofti* filariasis in New Guinea.

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SKIN TESTING FOR FILARIASIS WITH AN HOMOLOGOUS ANTIGEN

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The desirability of greater specificity in the serologic diagnosis of such diseases as filariasis has long been recognized. Unless a microfilaraemia is present, reliance must be placed upon a syndrome suggestive of filariasis aided by some indirect method such as complement fixation, precipitin reaction, or skin testing. While antigenic extracts of the microfilariae and adults of the dog heartworm, *Dirofilaria immitis*, and others such as *Dracunculus medinensis*, *Setaria equina* have proven of some value (Bozicevich and Hutter, 1944; King, 1944; Oliver-González and Morales, 1945; Franks, Chenoweth, and Stoll, 1947; Huard and Tran Ank, 1950; Casile and Saccharin, 1953; Floch, 1954, 1955; Jachowski and Otto, 1955; Okabe and Matsuse, 1956; Thooris, *et al.*, 1956), it was hoped that an homologous antigen prepared from human microfilariae would provide an even more accurate diagnostic tool. A preliminary report of a portion of this work appears elsewhere (Hunter, Bozicevich and Warren, 1945; Warren, Warren and Hunter, 1946).

MATERIALS AND METHODS

The method of preparing the antigenic extract of microfilariae of *W. bancrofti* was essentially the same as that described by Bozicevich and Hutter (1944) and Warren, Warren and Hunter (1946).

This filarial antigen was tested initially in dilutions of 1:4000, 1:8000, 1:12000, 1:16000 and 1:20000, while the leukocyte-stroma con-

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trol was employed at a dilution of 1:4000 in all individuals. The best reactions were secured with dilutions of 1:8000 which was selected for testing.

In order to raise the smallest possible wheal, test individuals were inoculated intradermally with approximately 0.01 ml of fluid. The volar surface of the forearm was used for these tests. Reactions were read within 10-15 minutes. A reaction was considered to be positive when the wheal produced by the antigen exceeded that of the control by at least 3 mm.

RESULTS

The antigen was tested on known negatives, known negatives with allergies, known negatives harboring intestinal helminths or malaria (*P. vivax*) and positives with a microfilariæmia. Some individuals from an endemic area suspected on clinical grounds of having filariasis together with others believed to be negative were also tested. The results are summarized in the Table, except those for this last group, which yielded equivocal results.

*Summary of tests with antigen made from microfilariæ of Wuchereria bancrofti (nocturnal periodicity)*¹

Groups tested	Number tested	Number	
		Positive	Negative
Non-exposed, non allergic			
Negative for helminths ²	78	0	78
Non-exposed, allergic			
Negative for helminths ³	42	4 *	38
Non-exposed			
Positive for <i>P. vivax</i> malaria ⁴	8	0	8
Non-exposed			
Positive for intestinal helminths ⁵	106	0	106
Positive for microfilariæ of <i>W. bancrofti</i> ⁶ . .	55	54	1
Positive for microfilariæ of <i>W. malayi</i> ⁷ . .	14	14	0

¹ This table includes data from Hunter, Bozicevich and Warren, 1945; and Warren, Warren and Hunter, 1946.

² Americans never out of United States; 38 of these were tested with 1:4000, 1:8000 and 1:12000 dilutions of the antigen, the remainder with a 1:8000 dilution of antigen; all were tested with a 1:4000 dilution of the leukocyte-stroma control.

³ Americans selected for sensitivity from those presenting themselves at an allergy clinic were tested with 1:4000, 1:8000 and 1:12000 dilutions of the antigen and 1:4000 of the control.

⁴ Americans.

DISCUSSION

It is generally believed that intradermal reactions to filarial antigens are only group specific and may be an indication of an infection that was active, quiescent or recently cured. Consequently, it is of considerable interest to find an antigen which appears to be quite specific for active cases, thus holding possible usefulness in individuals where lymphangitis and elephantiasis are encountered.

The results secured with the various groups are summarized in the Table and need little additional comment. It appears that «normal» persons who are not known to be hypersensitive reacted negatively and that in a selected group of hypersensitive Americans the number of false positives was relatively low (4 of 42). It is also significant that there was only one false negative and no false positive reactions in the test individuals who showed a microfilaraemia (*W. bancrofti* or *W. malayi*). In contrast, Franks *et al.* (1947) reported that most of the negative reactions to the microfilarial *D. immitis* antigen tested by them was found in Okinawans who had a microfilaraemia due to *W. bancrofti*. This discrepancy cannot be explained satisfactorily at this time.

It is difficult to evaluate filarial skin testing antigens as diagnostic aids. This is especially true since the clinical diagnosis of many patients returned to the United States as having filariasis was uncertain. For a positive clinical diagnosis of this disease in the case of these individuals reliance had to be placed upon a history of residency in an endemic area, exposure to mosquitoes, together with reported attacks of adenitis, retrograde lymphangitis and scrotal swelling, apparently due to no other cause. Accurate evaluation of such data was doubly difficult because of an anxiety complex on the part of the patients. Coggeshall (1946) pointed this out when he reported no

5 Filipinos, 38; Japanese, 37; Koreans, 10; Americans, 21. The following helminths were encountered: *Ascaris lumbricoides* in 82; *Trichuris trichiura* in 76; *Strongyloides stercoralis* in 6; hookworm in 72; *Schistosoma japonicum* in 11; *Hymenolepis nana* in 2.

6 Americans: non-periodic 1, periodic 1; Filipinos, 30; Okinawans, 23. Some of these individuals served as controls for those tested in «5» to insure potency of the antigen being used.

7 Koreans.

* These were highly sensitive to foreign proteins when tested: (1) gave a 5×5 mm wheal at a 1:4000 dilution of *W. bancrofti* antigen and no reaction at 1:8000 or 1:12000; (2) gave a 6×5 mm wheal at all dilutions; (3) gave an 8×8 mm wheal at 1:8000; and (4) an 8×7 mm wheal at 1:4000 only.

significant difference of the lymphadenopathy in 200 persons diagnosed as having filariasis and 271 with malaria, although both groups did possess larger lymph nodes than the 98 men of the control group who had never seen tropical service. Furthermore he found no difference in the size of an appendage after exercise in one group diagnosed as having filariasis and in another group composed of normal individuals.

In addition to those who were tested in the Table a group of approximately 60 persons, supposedly exposed to Bancroftian filariasis, were divided into positive and negative groups by clinicians about a year later. These individuals were then subjected to intradermal tests with a 1:8000 dilution of antigen from the microfilariae of *W. bancrofti*. Unfortunately, the results were equivocal. It cannot be stated positively whether or not these persons had filariasis since a microfilaraemia did not develop in most. Therefore, one cannot tell if the fault lay in the tentative clinical diagnosis or in a lack of specificity of the antigen. In view of the apparent antigenic specificity as seen in the Table, one is inclined to speculate that the difficulty may have been in the clinical diagnosis.

Huard and Tran Ank (1950) found adult *D. immitis* antigen to be 60 to 80 percent reliable when tested against known positives and negatives.

Casile and Saccharin (1953) secured a positive intradermal reaction in 29 of 31 persons with a microfilaraemia using antigen from adult *Dracunculus medinensis*.

Unfortunately Floch (1954, 1955), using similar antigen, was unable to confirm their results and found only a 64 percent correlation with known positives and negatives in a group of 58, and only a 58 percent correlation in 55 of the same group using antigen from adult *Dirofilaria immitis*.

Such discrepancies are difficult to understand and point up the fundamental question of reliability. The present paper points out what appear to be discrepancies between the clinical diagnosis in the absence of a microfilaraemia and positive intradermal reactions with a 1:8000 dilution of antigen from the microfilariae of *W. bancrofti*. It is only the high degree of reliability of the test on proven positives and the low incidence of false reactions that justifies the suggestion that further tests be made in an endemic area where material is abundant and a more accurate evaluation can be made. Then perhaps it will

be possible also to determine the usefulness of such a test in detecting early filariasis before a microfilaraemia is established and to study its specificity in cases of single or mixed filarial infections with such worms as *Mansonella ozzardi*, *Acanthocheilonema perstans* or *Loa loa*.

SUMMARY

Persons infected and uninfected with helminths, malaria or microfilariae of *Wuchereria* sp. were tested intradermally with an antigen made from microfilariae of *W. bancrofti*. False reactions were few.

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AGGLUTINATION OF MICROFILARIAE IN THE BLOOD OF THE DOG INFECTED WITH *DIROFILARIA IMMITIS*

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Liu and Bang (1950) described precipitate around the cercariae of *Schistosoma mansoni* and their agglutinins in incubated immune serum. Incidentally, cercarial agglutinins were also noted in monkeys recently infected with *Trichinella*, hence not unique to schistosome worm. Standen (1952) recorded that normal cattle sera agglutinated the cercariae of *Schistosoma mansoni*. Stirewalt and Evans (1955) found that agglutination of cercariae was correlated to CHR reaction. Oliver-González *et al.* (1955) also demonstrated agglutinins against adult, cercariae and ova of *Schistosoma mansoni* besides precipitins in immune sera of the mammalian hosts.

The present report on agglutinins was observed in the routine laboratory diagnosis for the microfilariae of *Dirofilaria immitis*, the common heart worm of the dog, and may be considered in the light of an extension of the above cited investigations. So far as observation carried in 20 dogs of both sexes, it appeared to be almost confined to the female subject and the phenomenon seemed most pronounced in pregnant individual. For the sake of brevity, a representative subject is hereto described. The citrated blood specimen was obtained from a pregnant bitch nearing the gestation period. It was kept overnight under room temperature in 5 cm³ vial. The following morning, the sample was stored in the refrigerator and kept under this condition for approximately two hours before microscopic examination was carried. Drops of blood specimen were made in slide, using a 0.5 mark of a Thoma pipette for red corpuscle and covered with cover slip.

The preparation was examined under low power objective of a microscope with 10 × eyepiece. The results are presented in the following table:

Clumps of microfilariae of Dirofilaria immitis

Slide number	Microfilariae													Free	Total
	Clumps number														
	1	2	3	4	5	6	7	8	9	10	11	12			
1.	2	19	21	20	—	—	—	—	—	—	—	—	217	279	
2.	1	31	6	15	24	1	1	—	—	—	—	—	188	267	
3.	6	16	48	9	6	4	7	—	—	—	—	—	57	153	
4.	1	4	8	8	1	—	—	—	—	—	—	—	89	111	
5.	3	3	15	5	2	3	2	7	—	—	—	—	32	72	
6.	50	3	21	28	22	3	39	53	4	71	43	4	34	377	
7.	11	43	16	11	2	—	—	—	—	—	—	—	23	106	
8.	4	6	3	23	5	3	3	—	—	—	—	—	33	90	
9.	12	4	2	28	18	3	—	—	—	—	—	—	47	114	
10.	8	19	7	28	6	60	3	5	—	—	—	—	48	184	

The present case under report had 49,400 microfilariae per cubic centimeter of blood. As shown in the Table, the number of clumps of microfilariae differs in different slides, ranging from 4 to as high as 12 clumps. By the same token, the number of microfilariae varies in different clumps, the range being from 1 to 71 microfilariae. In general, the larger the number of microfilariae involved in the different clumps, the lesser is the number of free microfilariae in the blood samples. From the different clumps examined, it was noted that the tail ends of the microfilariae appeared initially involved in the clumping process, while the anterior ends remained free, projecting more or less radially from the core of the clump (Fig. 1). In those clumps examined from one to five days after the blood sample was secured, most of the microfilariae were active and observed attempting futilely to extricate themselves by wiggling away from the agglutinated mass. In some, the center of the clump consisted of dead microfilariae arranged in most irregular pattern in the mass of an apparently homogenous substance (Fig. 2). In stained preparation, the mass appeared finely gran-



Fig. 1 — Agglutinated microfilariae in citrated blood. $\times 140$

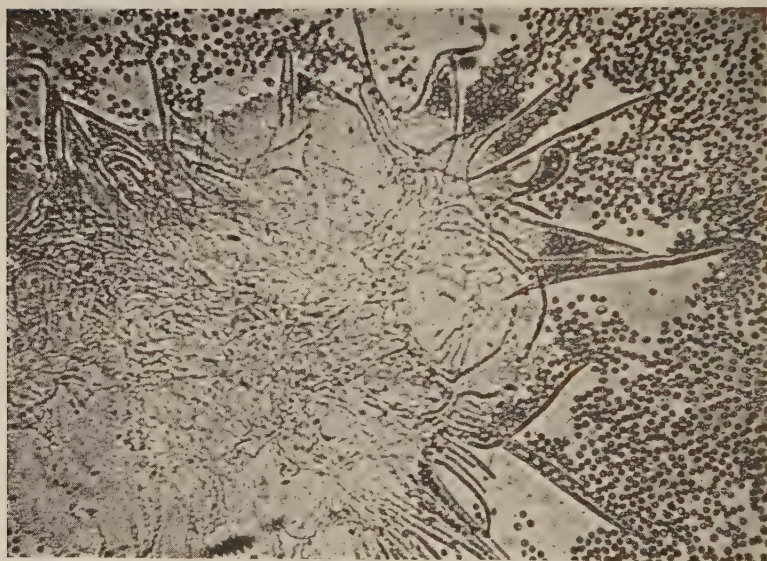


Fig. 2 — Agglutinated microfilariae in citrated blood with core consisting of microfilariae arranged irregularly. $\times 140$

ular and took blue dye from methylene blue, Wright's or Giemsa stains. While most of the clumps were circular, a number elliptical to irregular in outline. The size of the clumps measured ranged from 0.1 mm to 0.7 mm in its widest diameter.

COMMENTS

The agglutination phenomenon reported in this paper appears to differ from the agglutinins observed in *Schistosoma mansoni* infection (loc. cit.), in the sense that it is almost confined to the female subject and most pronounced in pregnant individual nearing the end of the gestation period. Moreover, the process follows more or less a rosette pattern similar to that observed in trypanosome infections.

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UMA DOENÇA NOVA EM GOA: A FILARIASE DE BANCROFT

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CONSIDERAÇÕES GERAIS

A filariase é uma doença conhecida desde remota antiguidade, mas a sua introdução na Índia Portuguesa é relativamente recente.

Largamente espalhada, sobretudo na zona intertropical, com foros de terceira moléstia parasitária na ordem de frequência, após o paludismo e a esquistossomíase, o seu estudo, todavia, foi por muito tempo relegado a um plano secundário, em consequência talvez de ser pouco dinâmica, pois raro prostra ou mata a sua vítima.

Actualmente, porém, já se vai reconhecendo a importância nosológica e social deste flagelo, pois, além da merecida atenção que lhe dedicam os estudiosos da medicina tropical, reuniu-se em 1951, em Tahiti (Oceânia Francesa), o primeiro congresso internacional consagrado exclusivamente à filariase e a Organização Mundial de Saúde promove e subsidia investigações sobre a sua epidemiologia, profilaxia e tratamento.

HISTÓRIA

Com o seu berço provável nas ilhas da Polinésia, a filariase teria sido levada, pelos mercadores que iam lá em busca de ouro e de pérolas, à Índia e ao Egipto e daí, pelas levas de escravos, à América. Na Índia espalhou-se largamente em tempos recuados, pois Sushruta, o conhecido médico aiurvédico de Benares, a descreveu em 600 a. C. tal como se manifesta na sua forma avançada: a elefantíase.

Há um quarto de século ela não figurava ainda na nosologia local senão, talvez, por algum raro caso de importação. Os esforços do nosso mestre e insigne microbiologista Prof. Froilano de Melo para demonstrar a sua existência entre nós, de que ele suspeitava, foram infrutíferos. Mais tarde o Dr. Sunder Rao, da Escola de Medicina Tropical de Calcutá, a pioneira de investigações nesse ramo de patologia na Índia, que cá esteve e colheu 2000 espécies de sangue, não logrou encontrar microfilárias em nenhum deles e ainda há seis anos, quando nessa cidade indiana frequentei o Curso de Higiene e Saúde Pública, se ensinava na cadeira de Epidemiologia, no capítulo da distribuição geográfica das filariases na Índia, fazendo-se, talvez, eco das conclusões do citado investigador, que essa doença era inexistente, entre outras regiões, em Goa, Damão e Diu.

Ultimamente clínicos e analistas vinham verificando, na capital do Estado, casos da doença filárica, mas coube ao Dr. Mortó Dessai, ao tempo assistente da Escola Médica, a prioridade na publicação dos quatro primeiros, confirmados por análises laboratoriais, dois deles autóctones.

O nosso caso não foi, porém, o único da penetração sub-reptícia da verminose numa área até aí indemne. Mesmo à nossa ilhargá, em algumas localidades dos estados indianos de Bombaim e de Saurashtra, mormente no litoral, estão a preencher-se paulatinamente, desde os alvares deste século, vastas clareiras que existiam, e na própria cidade de Bombaim a doença assentou arraiais pelo menos em dois quarteirões: o de Bandrá e o de Mahim.

CAMPANHA CONTRA A FILARÍASE

Dado o alarme pela publicação dos primeiros casos, os Serviços de Saúde procuraram organizar uma campanha contra a investida do parasita, que, embora com interrupções, me esteve confiada até aos primeiros meses do ano findo de 1957.

Iniciada em meados de 1953, essa campanha orientou as suas actividades em três direcções: 1.^a, inquérito epidemiológico; 2.^a, serviços clínicos aos doentes; 3.^a, profilaxia.

O inquérito epidemiológico para apurar a incidência, a natureza e a extensão da doença abrangeu os três elementos do primeiro termo

da equação filárica: homem infectado+agente vector+homem receptivo=infecção. O reservatório de vírus era constituído naturalmente pelo homem doente e foi nele que se fizeram as primeiras tentativas para se descobrir as microfilárias, mas sem êxito, excepto num caso.

Quando me foi entregue este serviço, em fins do mesmo ano, ciente de que em zonas de endemicidade filárica a percentagem da microfilaremia é sempre bem mais elevada na população aparentemente sã que na doente, dirigi os trabalhos no sentido da pesquisa da microfilária no *entourage* dos doentes e rapidamente se foram descobrindo muitos casos de doença inaparente, verdadeiros porta-germes, os mais perigosos e importantes de conhecer do ponto de vista sanitário.

Assim, reconhecida a origem filárica da doença e identificada a espécie, pelos caracteres morfológicos e pela mensuração à câmara clara, como *Wuchereria bancrofti*, a prospecção dos infestados e o seu tratamento para fins profilácticos — redução do reservatório de vírus — foi o trabalho principal. Ao mesmo tempo foram estudados, diagnosticados e tratados todos os casos de doença manifesta de que se teve conhecimento e procedeu-se à investigação da fauna mosquítica local no sentido de se descobrir o transmissor e tentar a sua redução ou eliminação.

Sabido que os factores meteorológicos condicionam a endemização da filariase e que a sua incidência é mais elevada nas zonas litorais dos entretrópicos e nas margens dos grandes rios, onde a temperatura e a humidade são suficientemente elevadas e constantes, mantendo-se a primeira entre 22°C e 35°C e a segunda entre 60 e 100 por cento, é indispensável conhecer a feição meteorológica da área infestada. Conquanto essa área seja extensa, compreendendo uma grande parte do território deste Estado, pois no curso do inquérito verificou-se a ocorrência de casos de transmissão local nas cidades de Margão, Mapuçá e Diu e em algumas aldeias limítrofes da capital, só a esta e à pequena aldeia fronteira de Reis-Magos, os dois focos mais importantes, se limitou a prospecção.

Assim, para melhor sistematização do assunto, dividi-lo-emos nos seguintes capítulos: 1) Fisiografia e clima da cidade de Goa; 2) Prospecção dos portadores de microfilárias; 3) Casos de doença filárica; diagnóstico e tratamento; 4) Fauna mosquítica e sua infestação; 5) Medidas profilácticas.

1) FISIOGRAFIA E CLIMA DA CIDADE DE GOA.

Situada na margem esquerda do rio Mandovi, junto da sua embocadura no oceano Índico, a 15° 30' de latitude N. e 73° 52' de longitude E., a cidade de Goa ocupa uma parte da planície que constitui a ilha do mesmo nome, com um relevo orográfico cuja altitude não excede 60 m, e é cortada por dois afluentes do mencionado rio Mandovi.

A sua população, segundo o último censo, é de 31 950 habitantes.

O regime de monções a que está sujeita dá ao seu clima feição especial, distinguindo-se no curso do ano três estações: uma estação quente, de Março a Maio, outra fresca e húmida, de Junho a Outubro, e a terceira, fresca e seca, de Novembro a Fevereiro. Os seguintes quadro e gráfico sintetizam os elementos meteorológicos mais importantes:

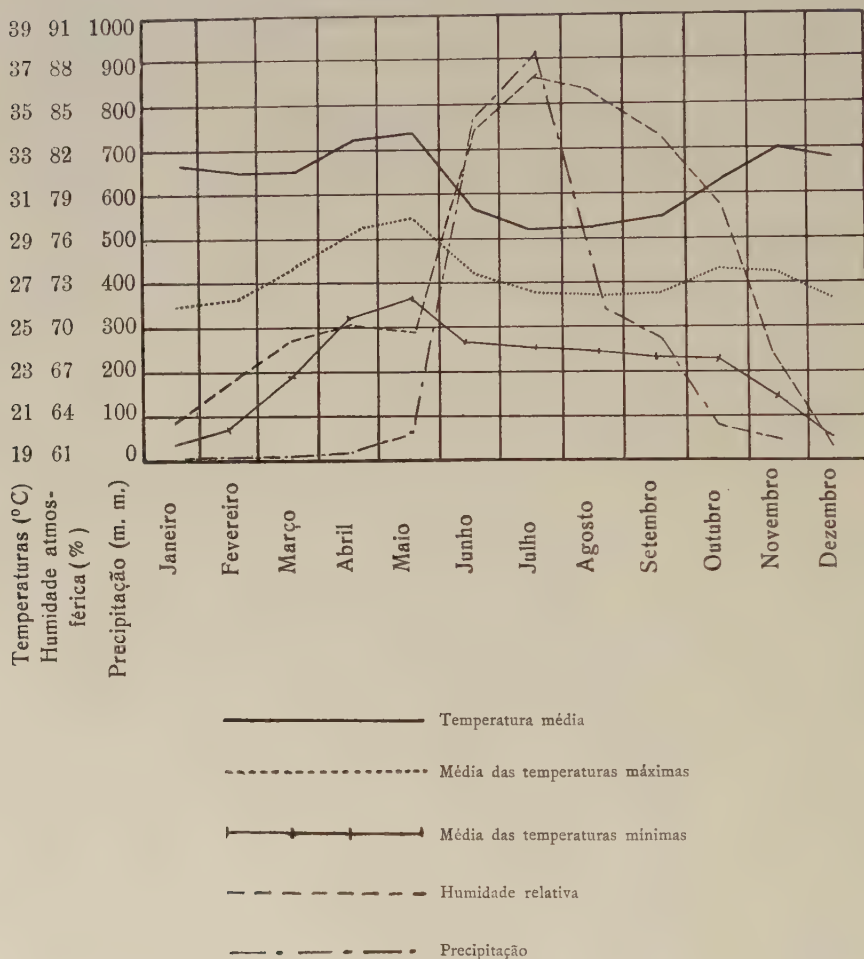
QUADRO 1 — Elementos climáticos da cidade de Goa (médias relativas a um período de 40 anos)

Meses e ano	Pressão atmosférica (mb)	Temperatura do ar (°C)					Humidade relativa (%)	Nebulosidade 0-10	Precipitação m. m.	
		Média			Absoluta				Total	Máxima em 24 horas
		Média	Máxima	Mínima	Máxima	Mínima				
Janeiro	1 009,4	26,0	32,3	19,8	36,2	14,8	63,5	1,6	0,4	1,1
Fevereiro	1 009,0	26,2	32,0	20,4	37,2	14,3	66,4	1,3	0,0	—
Março	1 007,9	27,7	32,0	22,8	39,0	16,4	69,2	2,1	0,1	0,8
Abril	1 006,3	29,3	33,4	25,2	36,9	20,8	70,1	3,5	12,7	23,5
Maio	1 003,5	30,0	33,7	26,4	36,0	21,6	69,9	4,7	61,3	50,6
Junho	1 002,7	27,4	30,5	24,4	35,6	20,0	83,4	8,1	761,1	191,4
Julho	1 002,9	26,7	29,3	24,1	32,0	20,9	86,9	8,4	914,2	141,4
Agosto	1 004,2	26,6	29,3	23,9	33,7	20,4	86,2	7,9	403,1	111,1
Setembro	1 005,5	26,7	29,8	23,6	33,0	21,0	83,4	7,2	287,5	96,9
Outubro	1 006,6	27,6	31,6	23,6	36,6	19,0	77,9	5,2	89,3	153,6
Novembro	1 007,7	27,4	33,0	21,9	37,1	15,9	67,4	3,5	55,1	64,5
Dezembro	1 009,4	26,4	32,8	20,1	36,3	14,1	61,3	2,4	2,5	6,0
Ano	1 006,3	27,4	31,7	23,0	39,0	14,1	73,8	4,6	2 587,3	191,4

(Extraído do «Panorama da amebíase na Índia Portuguesa», por Almerindo Lessa *et al.*, in *Bol. Cl. e Estat. do Hospital do Ultramar*)

Como se vê neste quadro, oito meses do ano são, pelas suas temperatura e humidade, propícios à evolução da filária, de maneira a completar o seu ciclo vital nos dois hóspedes, intermediário e defini-

1 — Gráfico dos elementos climáticos de Goa



tivo, e à proliferação do mosquito, com a ressalva de alguma redução deste no ácupe da época pluviosa, em que as copiosas precipitações da monção varrem as larvas.

A estes factores naturais juntam-se outros criados pela mão humana, que convertem a cidade num paraíso de mosquitos, especialmente a drenagem deficiente das águas domésticas através de uma rede de esgotos antiquada, imprópria e mal conservada.

São quase as mesmas as condições meteoro-telúricas da aldeia de Reis-Magos, que fica na margem oposta do Mandovi, resvês à cidade.

2) PROSPECÇÃO DOS PORTADORES DE MICROFILÁRIAS.

Com condições climáticas favoráveis e hóspede intermediário em abundância, faltava apenas a semente para que, cultivada em terreno receptivo — que é, afinal, todo o homem são —, germinasse, dando farta messe. E ela chegou um dia, albergada no organismo de algum infectado procedente das zonas de endemicidade filárica da Índia ou de África, talvez mesmo algum filho de Goa que por aí andou mourejando longos anos para ganhar a vida, e que o mosquito se encarregou de transplantar para as pessoas vivendo em sua volta.

Endemizou-se assim a doença, constituindo-se o reservatório de vírus local, que gradualmente cresceu por novas transições e pelo acréscimo de portadores vindos de zonas infectadas, devendo-se salientar entre estes as tropas africanas, nas quais, sobretudo nas procedentes da província de Moçambique, a incidência de infecção é elevada.

Nestas tropas verificou-se também a existência de microfilárias de *Loa loa* e de *Acantocheilonema perstans*, mas este facto não representa para nós problema sanitário, visto não existirem aqui os hospedes intermediários destes vermes.

A prospecção dos portadores de microfilárias efectuou-se pela análise sistemática do sangue de toda a população da cidade. A colheita por picada de dedo foi feita entre as 22 e 1 horas, andando-se de porta em porta após prévia propaganda pela imprensa e pela rádio e aviso no próprio dia ao chefe da família. Não obstante, muitas portas se nos fecharam na cara, regressando por vezes, após horas de labor nocturno, com uma dezena de esfregaços, o que obrigou os Serviços de Saúde a promulgarem um diploma legislativo impondo sanções a quem se recusasse a deixar colher sangue para análise.

Dada a dificuldade, nas condições em que se fazia a colheita — de noite e fora do laboratório —, do exame a fresco, aliás mais simples, adoptou-se o método de gota espessa examinada ulteriormente no gabinete depois de desmoglobinizada a água destilada e corada a *Leishman*. Cada preparação era feita com três gotas grossas ou quatro mais pequenas de sangue, ocupando, depois de estendidas, cerca de um terço da lâmina, quantidade que verifiquei corresponder aproximadamente a 60 mm³, e contado o número de microfilárias encontradas para os efeitos de se apreciar, *grosso modo*, a capacidade infectante para o mosquito e o grau de redução após o tratamento. Usei também, mas só em alguns casos de doença manifesta em que o exame de gota espessa foi negativo, o método de concentração a formol ou ácido acético, mas o resultado foi sempre igualmente negativo.

Pretendi usar a intradermoreacção ao antigénio filárico para julgar da sua utilidade na prospecção e, como não o tínhamos cá, consultei a Escola de Medicina Tropical de Calcutá e o Instituto Haffkin, de Bombaim, que responderam que não o preparavam, mas a referida Escola de Calcutá informou que o Instituto Guindy, de Madrastra, talvez estivesse habilitado a fornecê-lo. Entrando em correspondência com o mesmo Instituto, este comprometeu-se a satisfazer a primeira requisição, que até foi paga, mas nisto agravou-se a tensão das relações com a Índia e a tentativa malogrou-se.

Eis alguns dados estatísticos sobre a prospecção dos portadores de microfilárias:

Número total dos indivíduos cujo sangue foi examinado, 9608.

Foram encontradas microfilárias em 512.

A taxa de microfilaremia foi, portanto, de 5,3 por cento.

Esta taxa não dá, porém, ideia precisa da incidência da doença, visto que foi tirada em grupo um tanto heterogêneo, pois, além do elemento local, que está mais sujeito a endemia pelo seu carácter de permanência, abrange outros étnicamente estranhos com residência em geral transitória, como são os militares expedicionários europeus e africanos.

Convém separá-los e, assim, esse total desdobra-se em:

Europeus: 405, com 1 positivo; taxa de microfilaremia, 0,2 por cento.

Africanos: 1656, com 111 positivos; taxa de microfilaremia, 0,7 por cento.

Naturais: 7456, com 400 positivos; taxa de microfilaremia, 5,4 por cento.

Para determinar a incidência da verminose temos de considerar todos os infectados, isto é, os doentes com sintomas, embora sem microfilárias no sangue, e os doentes assintomáticos. Os enfermos arrolados cujo sangue foi negativo para microfilárias são em número de 233. Destes, alguns são de fora da cidade, mas em contrapartida haverá doentes na cidade dos quais não tivemos conhecimento, de forma que esse número pode ser considerado aproximadamente como o dos amicrofiláricos existentes em Pangim. A prospecção incidiu, no entretanto, em cerca de um quarto da população da cidade. Juntando ao número de enfermos inaparentes, que é de 400, um quarto da cifra dos da doença manifesta e com o sangue negativo, ou seja 58, teremos, *grosso modo*, o número total dos infectados, que passa a ser, assim, de 458 por 7454 pessoas, o que dá uma incidência global de 6,1 por cento, que faz situar a nossa cidade na zona de endemicidade fraca.

Discriminemos os naturais examinados segundo o sexo:

Dentre 3831 do sexo masculino são positivos 229. Percentagem de infestação, 6 por cento.

Dentre 3623 do sexo feminino são positivos 171. Percentagem de infestação, 4,7 por cento.

A infecção latente é, pois, mais frequente nos homens do que nas mulheres. Veremos logo que sucede exactamente o contrário na infecção manifesta, que afecta mais o sexo feminino.

A discriminação confessional da população nativa revela que dos:

4206 cristãos apresentam microfilárias 169, ou seja 4 por cento.

2495 hindus apresentam microfilárias 208, ou seja 8,3 por cento.

273 maometanos apresentam microfilárias 23, ou seja 8,4 por cento.

A incidência é, portanto, mais elevada nos maometanos e hindus, que têm, de uma forma geral, um *standard* higiénico e nutritivo mais baixo do que os cristãos.

No tocante à idade dos infestados, nota-se que o mais novo tem 4 anos e o mais idoso 83 e que as cifras e percentagens por grupos etários decenais são os seguintes:

10	anos:	23	positivos;	percentagem de incidência,	6.
20	anos:	113	positivos;	percentagem de incidência,	28.
30	anos:	75	positivos;	percentagem de incidência,	19.
40	anos:	64	positivos;	percentagem de incidência,	16.
50	anos:	49	positivos;	percentagem de incidência,	12.
60	anos:	43	positivos;	percentagem de incidência,	11.
60+	anos:	33	positivos;	percentagem de incidência,	8.

Vê-se, pois, que a incidência atinge o ápice na segunda década da vida e vai depois gradualmente baixando, porque em muitos deles a moléstia, de latente, se torna manifesta pela vida fora, desaparecendo-lhes as microfilárias do sangue.

Considerando a topografia da cidade, não há ponto algum dela que se possa chamar indemne, mas, incontestavelmente, a distribuição não é uniforme. Os bairros mais atingidos são os de Boca de Vaca, Fontainhas e Santa Inês, onde a infestação deve andar à roda de 10 por cento.

A prospecção na aldeia de Reis-Magos revelou a existência de 12 portadores de microfilárias.

Sendo, segundo os patologistas, as cifras de 12 e 300 microfilárias por 20 mm³ de sangue os limiares mínimo e máximo de transmissibilidade, verificou-se que, dentre os 512 portadores de microfilárias descobertos pela prospecção, 228 (45 por cento), ou seja, pouco menos de metade, podem ser considerados como inócuos e 284 (55 por cento) como contaminantes ao mosquito, 65 deles (23 por cento) em grau ótimo — com 100 a 200 microfilárias por 20 mm³ de sangue.

Nas famílias em que se efectuou a prospecção contam-se 2 com 6 membros infectados entre doentes e porta-germes, 2 com 5, 9 com 4, 12 com 3 e 60 com 2.

As microfilárias revelaram periodicidade nocturna, sendo, porém, por vezes, observadas mesmo em colheita feita de dia quando a infestação era em massa.

Em nenhum nativo portador de microfilárias encontrei adenite supra-epitrocliana, que Buxton verificou em 75 por cento dos infecta-

dos na Malaia e que, segundo vários autores, é também frequente nas zonas endêmicas africanas.

3) CASOS DE DOENÇA FILÁRICA; DIAGNÓSTICO E TRATAMENTO.

Na evolução da infecção filárica no homem distinguem-se 3 períodos: 1.º O alérgico, que vai desde a entrada do verme no organismo até à sua fixação no sistema linfático e maturação e é apenas denunciado pelo desequilíbrio coloidal provocado pelas proteínas do verme e traduzido em urticária, asma, funiculite, orquiepididimite, etc.; manifestações estas que quase nunca são ligadas à sua verdadeira causa; 2.º O da doença assintomática, revelada, além das reacções alérgicas, pelo aparecimento de microfilárias no sangue quando duma análise accidental ou sistemática; 3.º A fase obstrutiva ou de doença manifesta, que, embora menos importante do ponto de vista sanitário, a campanha não podia descurar, sob pena de malogro por desinteresse do público menos culto; assim, um dos pontos do seu programa foi o diagnóstico e tratamento dos filariosos.

No início da campanha andámos à procura dos doentes; mais tarde, após propaganda, os doentes passaram a vir à consulta por sua própria iniciativa ou mandados pelos clínicos, tendo já boa parte deles sido tratados em consultórios ou hospitais sob etiquetas várias: reumatismo, flebite, insuficiência cardíaca, doença venérea, etc.

É interessante a história do primeiro caso da nossa estatística na ordem cronológica e um dos mais antigos da série: cavalheiro hindu contando hoje 45 anos de idade e que até à altura em que se manifestaram os primeiros sintomas, 17 anos, nunca havia saído de Goa. Após uns pródromos que consistiam em dores lombares e de cabeça e apareciam de vez em quando, teve um acesso febril com calafrios que durou dois dias e após o qual notou intumescimento dos gânglios da virilha esquerda. Os acessos piréticos repetiram-se com intervalos de dois a três meses e a adenopatia, que se tornou bilateral, foi-se acentuando. Tratado aqui como um caso de doença venérea, sem resultado, seguiu para o hospital da Missão Americana de Miraj (Índia), onde, feito o diagnóstico de filariase, foi submetido a um tratamento médico e cirúrgico com remoção dos gânglios hipertrofiados.

Aparentemente curado e gozando de boa saúde, viu, volvidos anos, reaparecer os acessos febris e a urina tornar-se espessa e leitosa. Nova

hesitação dos clínicos locais e nova viagem do doente a Miraj, onde, diagnosticada a quilúria, foi esta devidamente tratada e curada e, apesar de há seis anos não se queixar de coisa alguma, o exame de sangue foi positivo para microfilárias. É o único doente na nossa casuística com adenolinfocele e quilúria e um dos poucos sem edemas e com microfilaremia.

A maioria dos casos foram de diagnóstico fácil. O síndrome clínico era bastante típico. Começa, por via de regra, por uma linfangite em placa ou em cordão, mais ou menos extensa, mais ou menos dolorosa, às vezes pruriginosa, na parte ântero-interna da coxa, geralmente acompanhada de adenite inguinal ou crural e, em muitos casos, de febre com calafrios, cefaleias, anorexia, náuseas, vômitos e edema considerável de todo o membro durante dois a três dias. A linfangite filárica, ao contrário da microbiana, segue uma marcha centrífuga ou retrógrada, isto é, desce da coxa à perna, localizando-se em geral no tornozelo. O edema, quase sempre duro, vai, após uma semana, em média, diminuindo e localizando-se nos maléolos e no fim de cerca de um mês fica apenas um ligeiro edema residual, indolor, que quase desaparece pelo repouso e aumenta um tanto após marcha prolongada.

Uns meses depois vem nova crise linfangítica, com o mesmo cortejo sintomático, e o edema residual que deixa é já mais acentuado. Repetindo-se as crises, o edema, com os sucessivos acréscimos e espessamento distrófico da pele, toma as proporções de paquidermia ou elefantíase. Na inflamação linfática podem enxertar-se micróbios vindos de alguma infecção focal e causar supurações e abscessos. Por vezes a linfangite e o edema surgem a seguir a uma contusão, levando a pensar numa osteoartrite traumática. Outras vezes sobrevêm durante a gravidez, pondo o problema do diagnóstico diferencial com a flebite.

A linfangite pode passar despercebida numa pele acobreada ou faltar de todo, sendo o edema o único sintoma.

O diagnóstico etiológico é, nestes casos, clinicamente difícil e o laboratório pouco adianta, pois em 97 por cento destes casos não se encontram microfilárias no sangue e a eosinofilia é inconstante. A existência de porta-germes na família ou no *entourage* dá algum peso à hipótese da origem filárica posta na ausência doutras causas que a expliquem.

As adenites agudas regressam em geral, sendo raro evoluírem para adenolinfocele. As manifestações genitais no homem são frequen-

tes: funiculites, orquiepididimites, hidrocele, sendo muito rara a elefantíase do escroto.

Eis algumas cifras colhidas da estatística sobre a maior ou menor frequência desses sintomas:

Número total de doentes verificados, 241.

O edema foi o sintoma quase geral em 235 casos e faltou apenas em 6 — 2,5 por cento.

Foi considerável (elefantíase) em 45 casos — 19 por cento.

Foi moderado em 77 casos — 32,7 por cento.

Foi ligeiro em 110 casos — 47 por cento.

Foi fugaz em 3 casos — 1,3 por cento.

Limitado a um membro inferior em 178 casos — 75,8 por cento.

Limitado a um membro superior em 16 casos — 6,8 por cento.

Estendendo-se aos dois membros inferiores em 33 casos — 14 por cento.

Estendendo-se a um membro superior e um inferior em 4 casos — 1,7 por cento.

Estendendo-se a um membro superior e aos dois inferiores em 3 casos — 1,3 por cento.

Errático, aparecendo na face, nuca, regiões glúteas ou membros, em 1 caso — 0,4 por cento.

Houve linfangite em 206 casos — 85,5 por cento.

Houve adenite em 105 casos — 43,5 por cento.

Houve crises linfangíticas febris em 113 casos — 47 por cento.

A linfangite deu lugar a supuração em 7 casos — 3 por cento.

Houve adenolinfocele inguinal em 2 casos — 0,8 por cento.

Houve quilúria em 1 caso — 0,4 por cento.

Em 68 doentes do sexo masculino sobrevieram lesões genitais:

Funiculite, orquiepididimite ou hidrocele em 23 casos — 34 por cento.

Elefantíase do escroto no início em 1 caso — 1,5 por cento.

Elefantíase do pênis e do escroto, moderada, em 1 caso — 1,5 por cento.

O exame de sangue para microfilárias foi positivo em 8 casos — 3,3 por cento.

Note-se que na população sã a prospecção deu uma taxa de microfilaremia quase dupla de 5,4 por cento, desproporção verificada, de resto, em todas as zonas de endemicidade.

A percentagem de eosinófilos na fórmula leucocitária tirada em 101 doentes foi a seguinte:

5 por cento ¹	em 8 casos —	8 por cento.	
10 por cento	em 35 casos —	35 por cento.	
15 por cento	em 24 casos —	24 por cento.	
20 por cento	em 15 casos —	15 por cento	} 33 por cento.
20 por cento+	em 18 casos —	18 por cento	

Como se vê, em 8 por cento dos doentes a taxa de eosinófilos é absolutamente normal. Em quase 60 por cento há eosinofilia inferior a 15 por cento, portanto incaracterística numa região onde as verminoses intestinais, que são frequentes, e a asma, cuja incidência não é desprezível, a originam também. Só num terço dos casos, ou seja, cerca de 33 por cento, é de alguma utilidade no diagnóstico. Para este fim, pois, a eosinofilia, como a microfilaremia, é teste de valor relativo. Elevada, é apreciável o seu contingente para o diagnóstico da doença; baixa ou normal, não a exclui, como não exclui também a ausência de microfíliarias no sangue enquanto a sua presença é patognomónica.

Apenas em 3 casos a percentagem de eosinófilos excede 40 e só um dos doentes, que tinha 60 por cento, se queixou de acessos ocasionais de tosse. Parece, pois, que aqui a filariase não desempenha papel importante na génese da eosinofilia tropical, ao contrário do que se verificou na Indonésia, onde se chegou à conclusão de que este síndrome se filia por vezes na infestação pela *Wuchereria malayi*. Todavia, é digna de ser arquivada a informação de um clínico local que verificou em alguns casos de síndrome de Loeffler uma baixa radical de eosinofilia com o tratamento pelo Hetrazan, mesmo na ausência de qualquer suspeita de infecção filárica.

Continuando a análise dos dados estatísticos, fluem ainda conclusões interessantes:

¹ Normal.

Quanto ao sexo:

Masculino: 68 casos — 28 por cento.

Feminino: 173 casos — 72 por cento.

Quanto à religião:

Cristãos: 141 casos — 58,5 por cento.

Hindus: 90 casos — 37,3 por cento.

Maometanos: 10 casos — 4,2 por cento.

Quanto à raça:

Europeus: 3 casos — 1,3 por cento.

Africanos: 1 caso — 0,5 por cento.

Naturais: 237 casos — 98,3 por cento.

Ao contrário do que se notava com os portadores de microfilárias — um ligeiro excesso dos do sexo masculino sobre os do feminino —,

II — *Gráfico comparativo da incidência segundo o sexo da doença filária latente e manifesta*



Incidência da doença filária latente



Incidência da doença filária manifesta

a doença manifesta é muito mais frequente, quase duas vezes e meia nas mulheres, como se vê melhor pelo respectivo gráfico. Atribuindo-se actualmente esta a um desequilíbrio da relação hóspede-parasita, este desequilíbrio surge talvez com mais facilidade na mulher. De resto, as estatísticas dos diferentes países são contraditórias neste ponto. A da Índia dá um excesso de incidência de 20 por cento para o masculino,

enquanto nas Guianas Britânicas o número das mulheres doentes é, como em Goa, maior que o dos homens, quase o dobro.

Divergência similar à que se nota no tocante ao sexo se verifica quanto à religião. Sendo quase iguais as comunidades cristã e hindu, há mais casos naquela, enquanto a percentagem dos portadores de microfilárias é mais elevada entre os hindus.

Entre os europeus aqui residentes encontrou-se apenas um portador-germes, um velho militar reformado, e 3 casos, um do sexo masculino, com permanência de cerca de 20 anos aqui e na União Indiana, e 2 do sexo feminino, que contraíram a doença 5 e 2 $\frac{1}{2}$ anos após a chegada. Entre os africanos vi apenas um caso, mas devem ter ocorrido mais.

Dentre as ocorrências nos naturais, numa grande maioria — 210 casos — a doença foi contraída nesta cidade — 87 por cento.

Em 21 casos a doença foi contraída em Reis-Magos — 8,7 por cento.

Em 7 casos a doença foi contraída em Mapuçá — 3 por cento.

Em 2 casos a doença foi contraída em Margão — 1 por cento.

Em 1 caso a doença foi contraída em Diu — 0,4 por cento.

Dos arredores de Pangim vieram 4 doentes, sendo dois da freguesia das Mercês, 1 de Taleigão e 1 de Santa Cruz, que só ocasionalmente passaram alguma noite na cidade, não se podendo tirar a limpo se a infecção teria sido contraída aqui ou naquelas aldeias.

O caso mais antigo da nossa estatística data de há 56 anos. É uma senhora asilada no Recolhimento da Serra, de 68 anos, que nos afirmou que o edema que tem na perna esquerda apareceu, com linfangite, adenite e febre, aos 12 anos de idade. Os restantes datam de há 30 anos ou menos.

Eis a discriminação dos casos segundo a idade da doença (tempo aproximado que decorreu desde os primeiros sintomas até à consulta):

1	ano:	112 — 46,5 por cento.
2	anos:	36 — 15 por cento.
5	anos:	37 — 15,1 por cento.
10	anos:	22 — 9,1 por cento.
20	anos:	28 — 11,6 por cento.
20+	anos:	6 — 2,5 por cento.

Comparando o número de doentes registados com a doença datando de menos de há 2 anos, temos as seguintes cifras:

1954 — 58.
1955 — 39.
1956 — 20.
1957 — 31.

que mostram que a incidência de infestação tendia a baixar, mas que o ano findo marcou nesta marcha regressiva uma excepção.

Observando a idade em que a doença se manifestou nos nossos 206 casos, podemos pelo seguinte quadro concluir que a incidência vai aumentando até os 30 anos e diminuindo daí em diante:

10	anos:	8 casos — 3,3 por cento.
15	anos:	40 casos — 16,6 por cento.
20	anos:	45 casos — 18,7 por cento.
30	anos:	59 casos — 24,5 por cento.
40	anos:	36 casos — 15 por cento.
50	anos:	28 casos — 11,6 por cento.
60	anos:	18 casos — 7,5 por cento.
60+	anos:	7 casos — 3 por cento.

Pelo gráfico III pode comparar-se a incidência segundo as idades da doença filárica latente e manifesta. Os mais novos são 3 crianças de 7 anos de idade e os mais idosos 2 velhos de cerca de 70.

É na época quente, de Março a Junho, que ocorre o maior número de casos novos de doença filárica e o agravamento dos antigos.

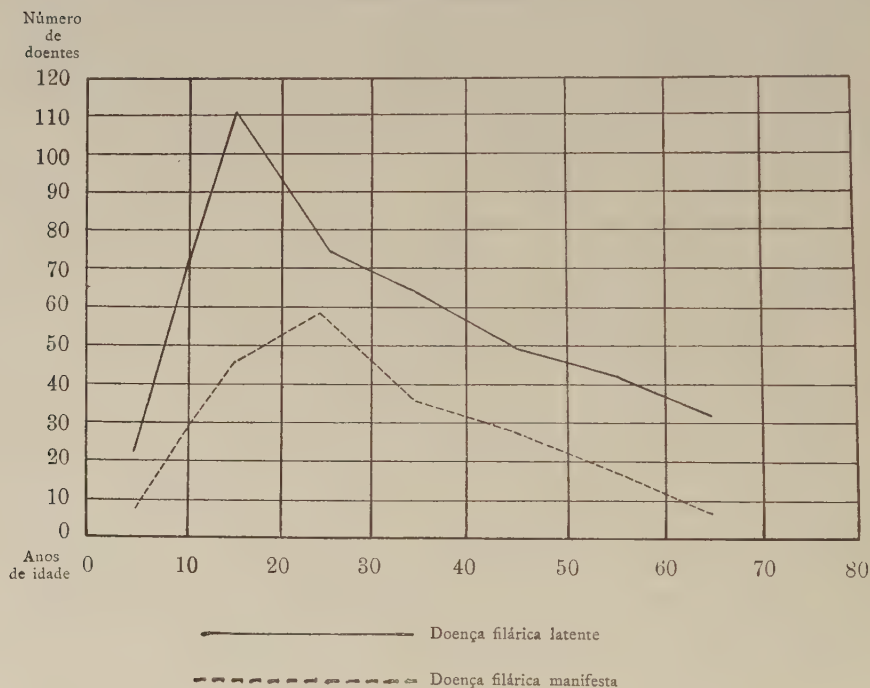
A filariase constitui, pelo estado de miséria fisiológica a que condena as suas vítimas, um problema de capital importância que preocupa os dirigentes dos países atingidos. Na União Indiana, por exemplo, as autoridades sanitárias, alarmadas com o incremento que a doença está a ter, projectam uma campanha nacional contra o que entendem ser o flagelo número dois, sendo o número um o paludismo.

De facto, embora a doença seja excepcionalmente fatal e não abaixe sensivelmente a duração média da vida, a sua expansão repercute-se de uma forma altamente nefasta não só na saúde pública, mas também no bem-estar e no avanço do país pelas perturbações psicos-

somáticas que origina nas suas vítimas e influência que elas exercem, sob o prisma económico e social, sobre a colectividade.

Trata-se, por exemplo, de uma criança doente, estudante ainda, a quem as crises febris, obrigando-a a faltar às aulas, tiram a oportunidade de ombrear nos estudos com os seus colegas, a quem às vezes a sua deformidade serve de tema para motejos. Nota que constitui

III — Gráfico comparativo da incidência segundo as idades de doença latente e manifesta



uma carga pesada para os pais, que têm de a levar de quando em quando ao médico para tratamento. Tudo isto gera na mente infantil um complexo de inferioridade.

Chega a idade núbil. A doença desfeia pernas elegantes e bem torneadas e marca com um estigma social particularmente as raparigas, que o procuram disfarçar usando os possíveis artifícios. Após o casamento, sobretudo tratando-se de uma localização genital da doença

e consequente esterilidade, vêm as dissensões no lar e a infelicidade na vida conjugal. Sendo chefe de família e com encargo de a sustentar, a doença inibe-o de trabalhar de uma forma efectiva, donde um desfalque nos proventos e uma vida doméstica cada vez mais trágica e miserável.

Em suma, é o ponto de partida de uma série de lutas no campo físico, moral e social, com a agravante de se reconhecer, após deambular por vários consultórios, que o mal, sobretudo nos períodos avançados, não tem cura, o que gera a revolta a princípio e depois, com o sentimento da derrota, a apatia e o fatalismo. E quando se atenta na multiplicidade de indivíduos nestas condições, torna-se patente que a doença emperra o progresso do país.

Não obstante novas armas terem sido forjadas nestes últimos anos no arsenal terapêutico das filariases, o tratamento mantém-se ainda em linhas empíricas e para o doente, mormente avançado, como são muitos casos observados aqui, é por vezes ilusório. As drogas consideradas como específicas não resolvem o problema. O Hetrazan, microfilaricida por excelência, se é de grande valor na profilaxia, é de resultados inconstantes como droga curativa, porque nem sempre actua na microfilária. As outras drogas, os arsenicais e antimoniais em uso — alguns novos produtos estão ainda na fase experimental, como a arsenamida e o melaminil fenilestibenato, ou MSb pela sua designação abreviada —, se a matam mais certamente, é no limiar das doses tóxicas. Mas mesmo que se logre matar as microfilárias, lá ficam nos linfáticos os seus cadáveres, que não podem ser eliminados e que — dizem quase unânimemente os patologistas — são, pelas toxinas provenientes da sua desintegração, mais perigosos que os vermes vivos. Todavia, as drogas específicas são receitadas *larga manu* sem embargo do contra-senso que implica o seu uso, uma vez que se aceite a concepção patogénica acima referida, porque se verifica praticamente que nos casos não avançados a doença estabiliza ou mesmo retrocede após a sua administração.

O esquema de tratamento por nós adoptado nos doentes em que o exame clínico e laboratorial dão certeza ou forte presunção de a etiologia das lesões ser filárica é o seguinte: uma cura de Hetrazan a 6 mg por quilograma de peso por dia, dividido em 3 doses, por

20 dias. Ao mesmo tempo, na fase aguda, havendo febre, prescrevo penicilina, procaina ou sulfas, além do repouso e de pensos húmidos locais de soluto saturado de sulfato de magnésio e, mais tarde, como a lesão mais constante e mais importante é o edema, recomendo localmente penso compressivo ou meia elástica, massagens e banhos. Se o edema desaparecer ou apresentar tendência para isso, como às vezes sucede, o doente fica em observação. Se persiste sem ou com pouca alteração, uso produtos arsenicais não tóxicos associados a vacinas, que actuam aqui acessoriamente como proteinoterapia específica, especializados por casas fabricantes indianas (arsenotifóide, filarina mista, etc.), pela via parentérica. A arsenotifóide, sobretudo, dá bons resultados, prevenindo linfangites e reduzindo edemas nos doentes em que o Hetrazan o não faça.

Em alguns casos alternei as séries dessas drogas com uma de antimonialis — fuadina ou antiomalina, por não haver no mercado o derivado considerado como mais eficaz, o Neostibosan —, mas, mesmo usados pela via intramuscular e em doses inferiores às recomendadas, deram às vezes reacções tóxicas. Empreguei também, em larga escala e com bons resultados, o fluoreto de sódio, recomendado por Subramaniam de Madrastra, sob a base de não se registarem casos de filariase nas localidades onde o teor de água em flúor é pouco mais alto que o normal. Embora lançado no mercado em empolas pela casa East-India sob o nome de Florocid, não usei esta especialidade, mas sim 1 cm³ de soluto a 1 por cento adicionado de novocaína ou álcool amílico preparado em frascos-empolas no seu laboratório e oferecido à campanha pelo Sr. Dr. Emidio Afonso.

Qualquer que seja, porém, o tratamento, o que dele se pode esperar é a cessação das crises linfangíticas e a diminuição ou mesmo o desaparecimento do edema quando não seja nem muito acentuado nem de muito tempo.

Nas elephantíases, por via de regra, não actua, limitando-se os resultados positivos, caso os haja, a pequena atenuação, como de resto é de prever, tratando-se de um processo escleroso e portanto irreversível. A glicerina em injecções intravenosas e intra-arteriais é a droga que melhor actua nas paquidermias. Não tive ocasião de usar o tratamento por injecções locais de hidrocortisona nem tão-pouco de recorrer à cirurgia.

Estatística dos doentes tratados na consulta externa:

1) Com *Hetrazan*: as crises linfangíticas desapareceram em regra. Quanto ao edema:

Lesões datando de	Número de casos	Desapareceu o edema	Diminuiu consideravelmente	Diminuiu pouco	No mesmo estado
Menos de 2 meses	44	32	12	—	—
Menos de 1 ano	67	13	47	10	—
Mais de 1 ano.	41	—	4	25	12
Soma	152	45	63	35	12

2) Com *filarina mista* (cacodilato de sódio+vacina mista T. A. B. antiestreptocócica e antiestafilocócica) da casa Bengal Immunity, de Calcutá: foram tratados 3 casos avançados, com ligeiras melhoras em 1, continuando os outros 2 no mesmo estado.

3) Com *arsenotifóide* (arsénico coloidal+vacina T. A. B.) da casa Calcutta Chemical Research Association: foram tratados 15 casos avançados com o seguinte resultado:

Edema bastante reduzido, 3.

Pequena diminuição do edema, 7.

Edema no mesmo estado, 5.

4) Com *antimoniais*: foram tratados 3 casos avançados, 1 com fuadina e 2 com antiomalina, sem resultado apreciável.

5) Com *fluoreto de sódio*: foram tratados 28 casos, sendo 12 recentes e 16 avançados, com o seguinte resultado:

Muito melhorados, 4.

Melhorados, 11.

Ligeiras melhoras, 5.

No mesmo estado, 8.

4) FAUNA MOSQUÍTICA E SUA INFESTAÇÃO.

O estudo do vector, elo indispensável na cadeia do ciclo filárico, implica o conhecimento da fauna mosquítica local, o seu *habitat*, a sua classificação e o grau da sua infestação, porque é sabido que a transmissão na espécie *W. bancrofti* é efectuada por um mosquito, em geral o *Culex fatigans*, com menos frequência dos géneros *Anopheles* e *Aedes*.

Este estudo incidiu sobre o insecto na fase adulta e larvar.

Em diversos pontos da cidade, em casas onde havia pessoas contaminantes ou não, foram apanhados mosquitos cujo número totalizou 235. Classificados, verificou-se que pertenciam ao:

Género *Culex*, 223 — 95 por cento.

Género *Anopheles*, 7 — 3 por cento.

Género *Aedes*, 5 — 2 por cento.

Se a diferenciação de géneros foi fácil, como a classificação dos anofelíneos que pertenciam à espécie *pseudopictus*, não pude levar a cabo a classificação em espécies dos culicídeos, por não mo permitirem os meus limitados conhecimentos de entomologia nem haver cá entomologista que me pudesse tirar de dificuldades. Verifiquei, todavia, que uma grande parte dos espécimes apresentavam os caracteres da espécie *Culex fatigans*.

Todos os exemplares foram dissecados, só um deles, um *Culex fatigans*, tendo sido encontrado infectado, o que dá uma percentagem de infestação de 0,4. Esta cifra é provisória, sujeita a correcção após estudo mais amplo e mais rigoroso por um entomologista. Fica por fazer o estudo da infestação laboratorial de mosquitos criados artificialmente, fazendo picar portadores de microfilárias para investigar mais pormenorizadamente a transmissão, o que requer um insectário e um técnico bem treinado.

O *survey*, a que se procedeu no início da campanha, das colecções aquáticas da cidade permitiu-me estudar o *habitat* larvar. Nas margens do rio Mandovi, do caneiro do Campal e do esteiro das Fontainhas não se encontraram larvas, naturalmente por a salinidade das águas lhes ser letal, mas em certos locais baixos e pantanosos (várzeas

das Fontainhas e palmares de Santa Inês), onde havia poças de água retida das chuvas ou acumulada por infiltração, foram encontradas larvas de culicídeos e anofelíneos. Estes focos, porém, são raros e limitados. Os mais importantes, e em que pululam quase exclusivamente larvas de *Culex* em profusão, podem ser divididos em três grupos: 1) rede de esgoto das águas pluviais e domésticas; 2) focos habitacionais; 3) fossas sépticas.

A rede de esgoto é de tipo arcaico, coeva da transferência da capital de Velha para Nova Goa, de secção quadrangular, de pedra laterítica não impermeabilizada, em parte aberta e, na parte fechada ou coberta, rota pela acção do tempo, das intempéries e dos roedores, que abriram aí galerias, parcialmente obstruída pelos detritos arrastados pelas águas da monção, sem o necessário declive nem bocas de inspecção e limpeza senão nos seus troços novos e que na estação seca nada drena, deixando, pelo contrário, estagnar águas sujas, que se convertem em verdadeiros viveiros de larvas, servindo também de abrigo ao mosquito adulto, que, saindo pelos ralos ao lusco-fusco vespertino, invade as habitações vizinhas e ao dealbar da manhã recolhe aos seus esconderijos, pelo instinto de retorno, e vai aí fazer a postura. Os canos ou sangrias abertas existentes em Boca de Vaca, Santa Inês e Fontainhas, contendo águas lamacentas semiestagnadas, com aparência de caldo de cultura de larvas, inundam esses bairros de mosquitos.

Outros criadouros importantes são as poças de água estagnada nos quintais das casas, por falta de livre drenagem dos escoadouros caseiros para o cano doméstico, que por vezes se faz através de uma sarjeta imunda, ligação defeituosa do cano doméstico com o colector ou obstrução dum ou doutro por cinzas usadas para arear os utensílios e outros detritos; chiqueiros resultantes do chafurdar dos suínos criados enquanto afrouxa a vigilância sanitária e camarária; latas vazias ou outros receptáculos atirados para lugares escusos, retendo água de chuva na época pós-monção, etc.

Um último foco localiza-se em melhoramento sanitário que, por desleixo, se converte em um incubador de mosquitos: as fossas sépticas, que atraem pelo cheiro de gases de decomposição as fêmeas para a postura através do tubo de escape ou dos interstícios entre as lajes de cobertura.

5) MEDIDAS PROFILÁCTICAS.

As medidas profilácticas a tomar na doença filárica fluem do estudo da equação já referida: homem infectante+insecto vector+, +homem susceptível=infecção.

Teòricamente, agindo em qualquer dos 3 elementos do primeiro termo, obvia-se à infecção, isto é, isolando ou esterilizando o homem infectante, destruindo o vector ou protegendo deste o homem são. Passando, porém, à prática, surge logo a primeira dificuldade. Não há um teste simples, cómodo e de confiança para se reconhecer com segurança o homem infectante. As cutirreacções feitas com extractos de filárias caninas dão resultados precários. O exame de sangue, que é o que mais garantias oferece, é incómodo, porque tem de ser feito de noite e carece de ser repetido, pois entre a contaminação e o aparecimento de microfilárias no sangue medeia um ano ou mais.

Há com certeza exagero na asserção de Norman Stoll de que numa zona endémica se deve considerar a incidência da infecção como sendo de 100 por cento, porque provar o contrário é difícil. Talvez haja vantagem em admitir nas regiões hiperendémicas, mas nas de grau moderado ou fraco de endemicidade a determinação do nível microfilárico da comunidade conserva todo o seu valor.

Uma vez conhecidas as pessoas infectantes pelo exame sistemático do sangue, não podemos, evidentemente, isolá-las como se procederia com um leproso ou com um tuberculoso, tratando-se, na sua quase totalidade, de indivíduos sem qualquer sintomatologia mórbida. O isolamento poderá limitar-se a mandar recolhê-las à noite dentro de um mosquiteiro; mas mesmo esse desiderato é de difícil consecução prática, dado o grande número delas. Podemos, porém, enveredar por outra alternativa, a esterilização dos infectantes, hoje possível com as armas que possuímos, se não total, pelo menos a redução do número das microfilárias no sangue abaixo do limiar da infectividade.

A destruição do vector, que o é também de várias outras doenças, deve incontestavelmente ser a medida príncipe, mas nem sempre é de execução fácil.

A terceira medida — protecção do homem susceptível contra a picada de agente transmissor — é naturalmente de escopo mais limitado. Entram aqui em acção os meios mecânicos de defesa individual

usados também contra a infecção palustre, como os repelentes, as redes mosquiteiras nas portas e nas janelas das casas de habitação, mosquiteiros nas camas, etc.

Na nossa cidade, abordando o problema da profilaxia da filariase, lançamos mão das duas medidas de maior alcance: redução do agente vector ou luta contra o mosquito; redução do reservatório de vírus pela hetrazanização dos portadores de microfilárias.

Redução do agente vector ou luta contra o mosquito.

Iniciada em 1954 com resultados satisfatórios, está ainda em curso, tendo-lhe por vezes faltado a continuidade e regularidade de operações absolutamente indispensáveis para o seu completo êxito.

Atacou-se o insecto nos seus três *habitats* preferidos: nas fossas sépticas mandou-se fechar a abertura distal dos tubos de escape com rede mosquiteira e os interstícios entre as lajes de cobertura com argamassa. Intensificou-se a actuação da policia sanitária na limpeza dos quintais, pátios e saguões das casas de habitação, eliminando na medida do possível os focos domésticos.

Quanto à rede de esgotos, enquanto ela não for substituída por outra obedecendo aos requisitos da engenharia sanitária, propusera à Câmara Municipal, para obviar às suas consequências nocivas, que se fizesse uma oclusão temporária, por meio de lajes de cimento armado amovíveis, dos ralos dos canos durante a estação seca, em que se efectua a estagnação — na época pluviosa ela é varrida pelas torrentes caudalosas da monção —, e se obrigassem os proprietários dos prédios a pôr em devido estado as ligações dos escoadouros domésticos com os colectores, intercalando um sifão, sistema que se poderia chamar de engarrafamento da população mosquítica, que não teria assim saída, ficando também barrada a entrada aos de fora para irem aos canos fazer a postura. Esta proposta, porém, por motivos vários, não se materializou, embora se tivessem já editado e publicado as respectivas posturas.

O que se fez de concreto foi a desobstrução e limpeza de alguns canos e sangrias, a regularização e impermeabilização do leito de uma delas e pulverização semanal de uma emulsão em água ou em malariol de pó dispersível de Gamexano a 6,5 por cento de isómero gama, na proporção aproximada de 25 g por litro, nos canos abertos, nos fecha-

dos, através de ralos, por meio de bombas manuais, e noutras colecções de águas domésticas ou pluviais. O seu efeito destruidor foi completado pela pulverização a quente, por meio de TIFA (Todd Insecticidal Fog Applicator), de solução de D. D. T. em petróleo ou gasóleo a 5 por cento ou, mais raras vezes, com fumigações de enxofre a Clayton.

O resultado deste combate ao mosquito foi animador, tendo-o reduzido em proporções muito apreciáveis.

Esterilização dos portadores de infecção latente.

A supressão ou, antes, a redução do reservatório de vírus, na espécie as microfilárias do sangue humano que o vector pudesse colher para transmitir à população sã, foi o segundo objectivo ao delinear a profilaxia da doença. Recorreu-se a uma droga introduzida na terapêutica em 1947 pela casa americana Lederle, sob o nome de Hetrazan, e hoje comercializada na Inglaterra sob o nome de Banocid e na França sob o de Notezine, um derivado de piperazina (citrato de 1-dietil carbamil-4-nutil piperazina), que é de comprovada eficácia como microfilaricida. A sua administração em dose conveniente — 6 mg/kg de peso/dia — reduz em mais de 90 por cento o número de microfilárias em 24 horas, e com maior rapidez nos que apresentam uma infestação maciça do que naqueles em que é moderada. Continuando por 10 a 20 dias, fá-las desaparecer, às vezes negatizando o sangue, mas, na maioria dos casos, há uma redução de 95 por cento ou mais, que dura, em média, quase 2 anos, período após o qual o número de microfilárias aumenta novamente, caindo mais uma vez se se voltar a administrar a droga. Ela não actua sobre as microfilárias que existem fora do sangue, por exemplo no líquido de hidrocele, e a sua acção sobre os vermes adultos, como se disse, é inconstante.

Penetrando mais intimamente no mecanismo da sua acção, verifica-se que não é, a bem dizer, microfilaricida, mas antes microfilarios-tático, sensibilizando como uma opsonina as microfilárias, que são destruídas nos capilares hepáticos pelo sistema reticulendotelial. Conquanto a sua eficácia destruidora não seja de 100 por cento, é uma arma de considerável valor, por consenso unânime entre os sanitaristas, no combate ao reservatório de vírus filárico, acrescendo que não é nada tóxica, nada alterável com variações climáticas e de manejo

fácil, pois se administra em comprimidos *per os*, necessitando apenas de vigilância para evitar fraudes, dada sobretudo a circunstância de causar em portadores com alto índice parasitário reacções desagradáveis, como febre, cefaleias, vômitos, devidos à destruição maciça de microfilárias, reacções que de resto podem ser bastante atenuadas pela administração simultânea de um anti-histamínico.

Dos 400 portadores de microfilárias naturais de Goa, 371 receberam tratamento, sendo 345 por um período mínimo de 7 dias. 16 fizeram tratamento incompleto, alegando reacção alérgica intensa após ingestão de Hetrazan, e 29 não foram tratados por se acharem ausentes das suas residências, onde foram algumas vezes procurados. Os porta-germes africanos, pertencentes na sua totalidade à tropa, foram tratados pelos médicos castrenses.

Em quase todos os portadores tratados repetimos o exame de sangue findo o tratamento, para verificar o resultado. Em cerca de 50 por cento as microfilárias desapareceram, mas noutros 50 por cento persistiu um número variável. Em alguns renovámos a análise após 8 meses a 1 ano e, mais uma vez, transcorridos 2 anos, verificando o acréscimo paulatino do número de microfilárias, só cerca de 10 por cento, continuando negativos após 2 anos. Noutro extremo, em cerca de 10 por cento também, o nível microfilárico, na sua ascensão, atingiu ou excedeu o primitivo. Nos 80 por cento intermédios o acréscimo foi variável, não tendo o número de microfilárias atingido a cifra inicial.

Embora auxiliar poderoso na profilaxia da filariase, não se pode contar com a hetrazanização, praticada isoladamente, para extinguir o reservatório de vírus e, portanto, erradicar a doença, porque, pelos motivos expostos e outros óbvios, é impossível descobrir todos os portadores de microfilárias e exercer sobre eles o *contrôle* necessário para extinguir a transmissão, permanecendo a destruição do vector a medida primacial.

Foi também esta a conclusão a que se chegou num estudo comparativo sobre o grau de eficiência das diversas medidas profilácticas usadas isoladamente a que se procedeu, desde 1950, na cidade de Cuttack e seus arredores, da província indiana de Orissa, de elevada incidência filárica, sob os auspícios do Instituto de Malariologia de Deli e o auxílio financeiro da O. M. S. Dividiu-se a área a sanear em zonas quase com a mesma extensão, população e condições climáticas

e em cada uma delas empregou-se exclusivamente uma medida: na primeira administrou-se o Hetrazan a todos os habitantes em massa, de uma única vez, durante 5 dias, na dose diária de 6 mg por quilo-grama de peso; na segunda fez-se uma campanha larvícida com soluto a 1 por cento de D. D. T. em malariol, espalhando-o semanalmente à superfície das colecções aquáticas; na terceira organizou-se a luta imagocida pela dedetização das paredes das habitações à razão de 200 mg por pé quadrado, 4 vezes no decurso do ano, e a quarta ficou sem nenhum tratamento para efeitos de comparação. Verificou-se que o resultado do primeiro método fora inicialmente espectacular, mas que os seus efeitos se esvaíam ao fim de 2 anos; o do segundo, se não tão rápido, foi mais duradouro, e o do terceiro foi medíocre, o que está de acordo com as verificações recentes de que o *Culex* adulto, que é o vector mais vulgar, é pouco sensível aos insecticidas residuais e cria rapidamente resistência a eles.

É escusado encarecer a vantagem da associação das duas medidas — luta antilarvar e hetrazanização — na nossa campanha, de que se devem esperar resultados mais satisfatórios em eficácia, rapidez e duração.

Direi de passagem que o tratamento dos portadores de microfilárias lhes não garante que a sua doença se não torne manifesta, de assintomática que era. Em 2 casos vimos sobrevir linfangites e edemas, apesar de 1 deles ter sido tratado a preceito durante 25 dias, mas sem que as microfilárias desaparecessem de todo.

Frank Hawking verificou que a arsenamida e o MSb administrados em injeção intravenosa diária, durante 15 dias, fazem desaparecer as microfilárias de uma forma definitiva pela morte dos vermes adultos, mas, além das reservas que a toxicidade dos produtos impõe, não é um tratamento que possa aplicar-se em massa, mesmo que as drogas sejam acessíveis.

CONCLUSÕES

1.^a A filariase é uma doença de introdução recente em Goa, datando de umas dezenas de anos.

2.^a A única espécie de filária encontrada na população local foi a *Wuchereria bancrofti*.

3.^a O vector é muito provavelmente o *Culex fatigans*, com *habitat* larvar mais importante na rede de esgotos, em más condições.

4.^a Os factores climáticos de Goa, sobretudo a temperatura, a humidade e a pluviosidade, são favoráveis à evolução do verme e proliferação do hópede intermediário, pelo menos 8 meses do ano.

5.^a A doença está espalhada pelas cidades mais importantes — Goa, Margão e Mapuçã —, sendo os focos de mais alta incidência a capital do Estado e a aldeia fronteira de Reis-Magos.

6.^a A incidência global da doença é de 6,1 por cento, devendo, portanto, considerar-se Goa como zona de endemicidade fraca.

7.^a A microfilaremia é de 5,4 por cento na população, sendo apenas de 3,3 por cento — quase metade — nos doentes.

8.^a Enquanto a infecção inaparente predomina no sexo masculino, a doença manifesta incide em grau muito mais elevado no feminino.

9.^a São sintomas mais frequentes da doença: edema, crises agudas de linfangite e adenite, com ou sem febre. A elefantíase, termo final — verdadeira cirrose cutânea —, atinge mais os membros inferiores que os superiores. Incidência das lesões genitais no sexo masculino em mais de um terço dos casos. Adenolinfocele, quilúria e elefantíases genitais muito mais raros.

10.^a Diagnóstico por vezes delicado quando a sintomatologia é incompleta. Os testes laboratoriais são de valor limitado. Não se pode contar com a microfilaremia por faltar em 97 por cento dos doentes. Eosinofilia superior a 15 por cento só em um terço dos casos.

11.^a Idade mínima na doença inaparente, 4 anos, e na doença manifesta, 7 anos.

12.^a Além das consequências de ordem nosológica, a doença tem repercussão social importante, pelas perturbações psicossomáticas que origina, bem como económica.

13.^a Sendo evidente, embora mais subtil é menos palpável que nas moléstias infecciosas, a expansão da doença na área infectada e para o resto do país, é incontestável a necessidade de uma campanha para cercear essa expansão com fito no objectivo final: a erradicação completa.

14.^a Enquanto não se construírem redes de esgoto, segundo os preceitos da engenharia sanitária, nas três cidades, é mister intensificar a luta sistemática contra o mosquito, medida basilar da campanha, garantindo-se-lhe a necessária continuidade.

15.^a Como medida acessória, deve-se continuar a desbastar o reservatório de vírus: *a)* pela pesquisa dos portadores de microfilárias na área endêmica e seu tratamento pelo Hetrazan; *b)* evitando o ingresso de novos portadores pelo exame, à entrada, das pessoas procedentes de zonas infectadas, especialmente as tropas africanas, e tratamento daqueles cujo sangue for positivo para microfilárias.

16.^a O tratamento é ainda conduzido sob base empírica, de resultado pouco satisfatório nos casos avançados de elefantíase, menos contingente nos casos recentes, em que a fibrose não se constituiu ainda. Agentes terapêuticos usados: Hetrazan, arsenicais, antimoniais, vacinas inespecíficas, fluoreto de sódio, glicerina, sulfas e antibióticos nas inflamações linfáticas agudas e fisioterapia.

NOTA PRELIMINAR SOBRE O CONTRÔLE QUIMIOTERÁPICO DA FILARIOSE BANCROFTIANA EM UMA LOCALIDADE DO SUL DO BRASIL

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Brasil

O grande transmissor da filariose bancroftiana no Brasil é o *Culex pipiens fatigans* (Rachou, 1956). O combate a este mosquito em nossos principais focos de bancroftose, quer em sua fase alada, quer em suas fases aquáticas, é complexo, difícil e de alto custo (Rachou, 1957a). Assim, dispondo de drogas derivadas da piperazina, praticamente atóxicas e de fácil administração por via oral, que reduzem rápida e acentuadamente a microfilaremia, e sabendo que, abaixo de certa densidade de microfilárias no sangue periférico, a infestação do mosquito vector é pequena, programamos uma prova de campo, empregando uma daquelas drogas como única arma de combate à filariose bancroftiana, a fim de avaliarmos a sua eficácia em uma campanha contra esta filariose.

O medicamento escolhido foi o Hetrazan, nome comercial do cloridrato de 1-dietilcarbamil-4-metilpiperazina, dos Laboratórios Lederle (American Cyanamid Co., Nova Iorque), já experimentado com objectivo semelhante em nosso país por M. Deane & Costa (1948), que, infelizmente, tiveram suas observações interrompidas, não chegando a conclusões definitivas.

Nossa prova está sendo feita em uma pequena localidade do estado de Santa Catarina, Ponta Grossa (município de Florianópolis), onde se sabia, por trabalhos anteriores (Ferreira e Ferraz, 1952a e b),

ser alta a incidência da bancroftose, traduzida por um índice de microfilaremia de 13,9 por cento, o mais alto do país. Iniciada em Dezembro de 1953, a nossa prova ainda não está terminada. Os resultados dos 4 primeiros anos de observação são aqui apresentados. Em obediência aos regulamentos destes Congressos, que limitam em 1500 o máximo de palavras das comunicações livres, apresentamos, e de modo sucinto, apenas os principais resultados preliminares.

DESCRIÇÃO SUMÁRIA DA PROVA

1.º Em Dezembro de 1953 realizámos um inquérito preliminar (Rachou *et al.*, 1955) para a actualização dos índices epidemiológicos, constando de:

- a) Colheita de sangue a domicílio do maior número possível de moradores, entre as 19 e as 23 horas; 3 amostras de 20 mm³ por pessoa. Hemoscopia e contagem de microfilárias.
- b) Capturas domiciliárias, com uma inspecção nocturna e outra matutina em cada casa. Identificação e dissecação dos mosquitos capturados; contagem das larvas de filária nos mosquitos infestados.
- c) Capturas extradomiciliárias com isca humana em 10 estações distribuídas pela localidade. Identificação e dissecação dos mosquitos capturados; contagem de larvas nos mosquitos infestados.

2.º Logo após o término desse inquérito, ainda em Dezembro de 1953, foi feita a medicação com Hetrazan, na dose diária de 6 mg por quilograma de peso corporal, durante 7 dias, dos portadores de microfilárias e dos elefantíasicos, ainda que sem microfilaremia patente.

3.º Inquéritos epidemiológicos iguais ao preliminar (1.º) foram feitos 3 (Março, 1954) e 6 (Junho, 1954) meses e 1 (Dezembro, 1954), 2 (Dezembro, 1955), 3 (Dezembro, 1956) e 4 (Dezembro, 1957) anos após a medicação.

INFORMES EPIDEMIOLÓGICOS

1.º A vila de Ponta Grossa, com 144 habitantes e 44 casas, das quais 37 habitadas (dados do inquérito preliminar), fica no extremo noroeste da ilha de Santa Catarina, que está enquadrada pelos paralelos 27º e 28º S. e meridianos 48º e 49º O. É uma localidade litorânea, de clima temperado iso-húmido. Temperatura média anual em torno de 20°C, média das máximas de 23,7°C e das mínimas de 17,8°C; 36°C foi a máxima já observada no município e 1,3°C a mínima. Janeiro a Março são os meses mais quentes, com a temperatura média em torno de 24°C; Junho a Agosto, os mais frios, com a temperatura média de 16°C. Pluviometria média anual de 1353 mm, mais ou menos uniformemente distribuída, com certa redução nos meses mais frios. Humidade relativa oscilando, mensalmente, entre 78 e 83 por cento, sendo a média anual de 81,6 por cento.

2.º O transmissor primário da bancroftose em Ponta Grossa é o *Culex pipiens fatigans*, mosquito de acentuada domesticidade, cuja densidade apresenta grandes oscilações estacionais, sendo alta nos meses mais quentes e reduzida nos meses frios (Rachou, Ferreira, Martins e Ferreira Neto, 1958).

3.º Em qualquer época do ano a *Wuchereria bancrofti* completa o seu ciclo evolutivo no *Culex pipiens fatigans*. A duração dessa fase extrínseca varia, no entanto, em muito com a temperatura, sendo bem mais curta no Verão do que no Inverno. Rachou (1957b) obteve na região um mínimo de 10 dias, em Fevereiro-Março, e de 32 dias, em Julho, para que a filária chegasse, no mosquito transmissor, ao terceiro estágio, à forma infestante.

4.º Estes dois factores, densidade do transmissor e duração da fase extrínseca da filária, autorizam-nos a admitir uma nítida variação estacional da transmissão da bancroftose em Ponta Grossa, sem que, no entanto, haja normalmente uma interrupção dessa transmissão no Inverno.

RESULTADOS PRELIMINARES DA PROVA

1.º No quadro junto apresentamos os dados relativos à microfilarémia e ao *Culex pipiens fatigans* em domicílio que nos parecem

Principais dados epidemiológicos da filariose bancroftiana em Ponta Grossa (Santa Catarina, Brasil) antes e depois do controle quimioterápico com Hetrazan (6 mg/quilograma corporal/7 dias)

Dados epidemiológicos	Antes do tratamento		Depois do tratamento					
	1953		1954			1955	1956	1957
	Dezembro		Março	Junho	Dezembro	Dezembro	Dezembro	Dezembro
Número de pessoas examinadas.	131		101	98	89	106	118	134
Número de pessoas com microfilárias	19		11	11	10	8	3	6
Índice de microfilaremia (por cento)	14,5		10,9	11,2	11,2	7,5	2,5	4,5
Número total de microfilárias	1 194		104	207	140	79	11	53
Microfilaremia média por lâmina examinada.	3,0		0,34	0,70	0,58	0,25	0,03	0,13
Microfilaremia média por lâmina positiva	21,7		3,9	8,6	5,8	5,0	1,6	3,3
Número de casas inspeccionadas	69		71	60	72	69	69	72
Número de casas com <i>fatigans</i>	45		46	21	48	48	28	34
Índice domiciliário (por cento)	65,2		64,8	35,0	66,4	69,6	40,6	47,2
Número de exemplares de <i>fatigans</i> capturados.	327		282	71	449	514	170	228
Densidade domiciliária.	4,7		4,0	1,2	6,2	7,4	2,5	3,2
Número de exemplares de <i>fatigans</i> dissecados	279		269	66	359	504	166	207
Número de exemplares de <i>fatigans</i> infestados	30		9	1	4	3	1	3
Índice de infestação (por cento)	10,8		3,2	1,5	1,1	0,6	0,6	1,4
Número de exemplares de <i>fatigans</i> infestantes	2		1	0	3	0	0	1
Índice infestante (por cento)	0,7		0,4	0	0,8	0	0	0,5
Número de larvas de todos os estádios	169		40	6	7	14	17	18
Média por exemplar dissecado	0,61		0,15	0,09	0,02	0,03	0,10	0,09
Número de larvas infestantes	14		3	0	4	0	0	7
Média por exemplar dissecado	0,05		0,01	0	0,01	0	0	0,01

suficientes para, em uma nota prévia como esta, se ter uma ideia sobre a eficácia da quimioterapia como arma única no *contrôle* da filariose bancroftiana.

2.º O índice de microfilaremia, isto é, o percentual de pessoas com microfilárias, sofreu, durante o primeiro ano após a medicação, uma pequena redução, já que de 14,5 por cento em Dezembro de 1953 caiu para 11,2 por cento em Dezembro de 1954; sua queda, porém, acentuou-se no segundo ano e, mais ainda, no terceiro, quando encontramos índices de 7,5 e 2,5 por cento respectivamente.

3.º A densidade de microfilárias caiu mais rápida e acentuadamente. A microfilaremia média por lâmina examinada passou de 3,0 antes da medicação para 0,53, 0,25 e 0,03 ao fim, respectivamente, do primeiro, do segundo e do terceiro ano após a medicação; os dados relativos à microfilaremia média por lâmina positiva foram de 21,7, 5,8, 5,0 e 1,6 nesses mesmos anos.

4.º A redução, anualmente progressiva, do índice de microfilaremia e da densidade de microfilárias nos autoriza a admitir, além da acção microfilaricida indirecta do medicamento, geralmente considerada, uma certa acção sobre as filárias adultas (filaricida ou lesiva dos órgãos de reprodução).

5.º No quarto ano após a medicação (Dezembro de 1957) o índice de microfilaremia e a microfilaremia média foram superiores aos do terceiro ano, embora ainda inferiores aos do segundo.

6.º Durante os 4 anos de prova as variações da densidade do mosquito transmissor não têm significação como um possível factor da redução observada na incidência da bancroftose.

7.º A infestação do *Culex pipiens fatigans*, traduzida pelos índices de infestação e infestante e pela densidade de larvas de filária, sofreu redução semelhante à da microfilaremia, devendo-se registrar que, nos inquéritos feitos ao término do segundo e do terceiro ano após a medicação, não foram encontrados exemplares com larvas infestantes, que, no entanto, compareceram no inquérito do quarto ano. O índice de infestação caiu de 10,8 por cento antes da medicação para 0,6 por cento no terceiro ano, subindo para 1,4 por cento no quarto; o índice infestante inicial, de 0,7 por cento, ainda era de 0,8 por cento ao fim do primeiro ano, para ser zero nos dois anos seguintes e de 0,5 por cento no quarto. Grande redução também foi observada na densidade de larvas de filária: de 0,61 antes da medicação, o número

médio de larvas por mosquito dissecado passou para 0,02, 0,03, 0,10 e 0,09 nos inquêritos seguintes à medicação.

8.º Após o quarto ano de observação, nova medicação, nas mesmas doses, foi feita aos indivíduos que acusaram microfilárias no sangue por ocasião do inquérito correspondente (Dezembro, 1957), estando programados novos inquêritos para a continuação das observações.

CONCLUSÕES

1.^a O Hetrazan dado aos portadores de microfilárias, na dose diária de 6 mg por quilograma de peso corporal durante 7 dias, reduziu a baixos índices a filariose bancroftiana em Ponta Grossa, até 4 anos após a sua administração, sem que aí fosse empregado qualquer método de luta ao mosquito transmissor.

2.^a Acreditamos que esse medicamento, por si só, administrado nesses mesmos moldes, de 3 em 3 anos, ou, para maior segurança, de 2 em 2 ou de 1 em 1, controle eficientemente a filariose bancroftiana, fazendo que esta deixe de ser um problema de saúde pública.

3.^a Procurando simplificar o *contrôle* quimioterápico — evitando um esquema de tratamento prolongado como esse — e torná-lo mais econômico em uma campanha em larga escala — suprimindo a hemoscopia rotineira para a descoberta dos portadores de microfilárias —, iniciámos em 1957 uma nova prova de campo em que o medicamento é dado em dose única (6 mg/quilograma corporal), de 6 em 6 meses, a todos os moradores da localidade, sejam eles filarióticos ou não.

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SUBSECTION 3

OTHER INFECTIONS

1ST SESSION: *Saturday, September 6th — 11:00 to 12:30 a.m. Room B*

The meeting was held under the chairmanship of Dr. Donald L. Augustine. The Rapporteur was Dr. W. H. Wright and the Secretary was Dr. F. A. Carvão Gomes.

The following papers were presented and discussed.

PROGRESS IN RESEARCH AND CONTROL OF THE HELMINTHIASES OTHER THAN SCHISTOSOMIASIS AND FILARIASIS

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The function of this Section is to consider the helminthic diseases other than schistosomiasis and the filariases. Appreciable advances have been made in our knowledge of these diseases during the interim period following the Fifth International Congresses in Istanbul in 1953. It is proposed to summarize briefly the major advances and to leave to the invited speakers on this program the task of presenting the details of the marked progress which has been made in this field.

HELMINTH PHYSIOLOGY

The physiology of animal parasites is an expanding field which is now receiving at least part of the attention which it deserves. Without adequate knowledge of the physiology of the parasitic agents

of disease, we cannot hope to cope adequately with the diseases which they produce and to treat and control these infections.

Bird and Deutsch (1) have investigated the nature of the cuticle of *Ascaris lumbricoides* and by the employment of X-ray diffraction and electron microscopy have confirmed previous findings that the outer integument is composed of 9 layers, viz. the outer cortical, inner cortical, fibrillar, homogeneous, boundary, outer fiber, middle fiber, inner fiber, and basal lamella layers.

In addition to conducting valuable research on the biochemistry and physiology of *Ascaris lumbricoides*, Fairbairn (2) has summarized recent knowledge of this subject. Some of the recent advances may be mentioned briefly.

With regard to the chemistry of *Ascaris*, Fairbairn and Passy (3) isolated trehalose, a non-reducing disaccharide, from fertilized *Ascaris* eggs, in which it comprised 7.9 percent of the decoated egg solids. Glycogen has been firmly established as the major carbohydrate constituent of the parasite and now paper chromatography has revealed the presence of only two low molecular weight carbohydrates, trehalose for one, with the other probably glucose. Concerning the lipids of *Ascaris*, Fairbairn (4, 5) has unequivocally confirmed the findings of Flury (6) that the volatile acids occur mainly as esters. Fairbairn (5) found that phospholipids comprised nearly 50 percent of the total lipids present in the muscle and cuticle. The ascaryl alcohol isolated by Flury (6) has been identified structurally by Polonsky *et al.* (7), and Fouquey *et al.* (8). Contributions have been made recently to the chemical nature of the *Ascaris* cuticle, although there is still doubt concerning the nature of the cortex protein.

Information has been added to the understanding of the carbohydrate metabolism of *Ascaris*. Two different oxidative systems may be present in *Ascaris* muscle. The properties of one of these, succinoxidase, suggest the presence of flavine enzymes which react directly with oxygen to form hydrogen peroxide. Preliminary evidence for the presence of flavinadeninedinucleotide (FAD) in a particulate succinoxidase preparation has been obtained by Bueding *et al.* (9), and FAD and other flavins were found in muscle by Yamao (10). The nature of the second system, which appears to be linked with the processes of oxidative phosphorylation, remains to be established. Later, Entner (11) demonstrated the presence of a partial pentose

phosphate pathway in *Ascaris* and has partially purified some of the enzymes.

Advances have been made in an understanding of the nitrogen metabolism of *Ascaris* and important findings have been forthcoming concerning the biochemistry of embryonation.

In studies on the chemistry and physiology of the human cestodes, Kent (12) isolated four different protein complexes from frozen *Hymenolepis diminuta* and established the amino acid composition of these complexes. Read (13) found that anaerobically no metabolic gas was produced by *H. diminuta* and that lactic acid was the major acid product excreted. Acid production was enhanced by the addition of glucose; galactose was utilized at a somewhat lower rate than glucose; but maltose, trehalose, lactose, and fructose were not utilized to a significant extent. The worm produced 31 percent more lactate under anaerobic conditions than under aerobiasis. Sharp differences were found in rates of acid production and glucose utilization by portions of worms divided linearly into sixths. In the latter connection, Daugherty and Taylor (14) demonstrated that the glycogen content of *H. diminuta* was high in the region nearest the scolex, but dropped in the next 10 cm, rose in the succeeding 10 cm to 20 cm, remained fairly constant, and finally declined in the last of the remaining gravid proglottids. It was theorized that the regional differences were due to certain aspects of intermediary carbohydrate metabolism. However, Daugherty (15) demonstrated active absorption of methionine by this parasite, indicating that the exterior surface is functionally more than a simple diffusion membrane.

With further respect to the carbohydrate metabolism of *H. diminuta*, Read and Rothman (16) reported that worms from hosts receiving dextrose or sucrose as the sole carbohydrate were markedly smaller than worms from hosts receiving starch as the only carbohydrate. When dextrans-maltose, an enzymatically degraded product of starch, was substituted as the carbohydrate component, the worms were much smaller than when glucose or sucrose was substituted. The mechanism of such differences in the relative qualities of different carbohydrates was not apparent.

In spite of the public health and medical importance of hydatid disease, little is known concerning the chemistry and physiology of *Echinococcus granulosus*. Recent studies have been reported by Agosin

et al. (17). The scolices were shown to comprise two polysaccharides, glycogen and another containing galactose and glucosamine. The presence of relatively large amounts of glycogen indicated that this carbohydrate is the major source of energy. The respiratory gaseous exchanges were definitely inhibited by fluoroacetate, but not by malonate, indicating possibly a terminal sequence resembling an incomplete Krebs cycle. The respiratory quotient was somewhat lower than in many other parasites. The aerobic respiration of the scolices was sensitive to cyanide in a degree sufficient to assume a marked dependency on heavy metal catalysis. However, there was no implication that the parasite possesses a cytochrome system. Finally, the scolices demonstrated a pronounced anaerobic CO_2 production, although the origin of this CO_2 remains to be elucidated.

Later, Agosin (18) found that, under aerobic conditions, glycogen is converted to a mixture of lactic, acetic, succinic, and pyruvic acids, and ethyl alcohol, with lactic acid being the most important end product quantitatively. Under anaerobic conditions, glycogen is fermented to a mixture of lactic, succinic, and acetic acids, as well as ethyl alcohol; lactic and succinic acids are quantitatively the most important end products.

With regard to the physiology of *Trichinella spiralis*, Agosin (19) demonstrated the presence of cytochrome-c, cytochrome oxidase, succinoxidase, and succinic dehydrogenase activity in homogenates and cell fractions of the third stage larvae. Goldberg (20) extended these observations and showed that spectroscopic analysis of intact larvae revealed the adsorption bands in the approximate positions of cytochrome c, b, and $a + a_3$. Goldberg concluded that the evidence indicated strongly that the cytochrome system for electron transport and the tricarboxylic acid cycle for terminal oxidative processes occur in the tissues of the third stage larvae of *T. spiralis*.

Recent studies have thrown some light on the mechanism of penetration of helminth larvae, although knowledge in this field is still relatively superficial. Lewert and his co-worker, Lee, have been active in investigating the nature of this phenomenon. It is largely through the work of these investigators (21, 22, 23) that it is known that some forms, i.e., the schistosomes and *Strongyloides*, possess enzymes which are proteolytic and collagenase-like in nature. These species are responsible for enzymatic alteration of the acellular, glyco-

protein, connective tissue barriers. At the same time, the species may possess mucopolysaccharidases and lipases. Other tissue penetrating forms, such as *Nippostrongylus*, are lacking the collagenase-like enzymes, and possibly mucopolysaccharidase, but do possess lipolytic substances. The penetration enzymes are not characteristic of all stages of the parasites, but in many forms are limited to those stages which penetrate the host.

In histochemical studies on the mode of penetration of the hexacanth embryos of *Taenia pisiformis* and *T. saginata*, Silverman and Maneely (24) found that the gland substance contained polysaccharide complexes and that the gland plays an active part in the penetration of the intestinal mucosa. The gland secretion appears to lyse the striated border, to loosen the ground substance between the epithelial cells, and to possess cytolytic properties which enable the embryo, anchored by its hooks, to penetrate between the epithelial cells into the connective tissue core of the villi.

While considerable progress has been achieved in efforts to elucidate the complex physiological mechanisms of helminth parasites of man, it is obvious that such efforts have been materially handicapped by inability to date to grow and maintain the helminths *in vitro* where enzymatic and other physiological processes can be observed and measured without the complicating influences prevailing in the host animal. Some start, however, has been made in this direction. Smyth (25) has successfully cultivated *Schistocephalus solidus*, a diphylobothriid cestode, *in vitro* and Weinstein and Jones (26) have accomplished the axenic development *in vitro* of *Nippostrongylus muris* to the adult stage, although final mating has not been achieved. These recent accomplishments offer promise that similar successes may soon be achieved with some of the helminth parasites of man. It is then that we can expect more rapid advances in the knowledge of the physiology of human helminths.

HOOKWORM ANEMIA

Thorson (27) reported that physiological saline extracts of the amphidial glands of *Ancylostoma caninum* increase the prothrombin time of dog plasma, and in addition the clot formed is not complete

when compared to those formed when saline extracts of the esophagus, or extracts of excretory glands are used instead of the amphidial gland extract.

Gerritsen *et al.* (28) employed radioactive iron in estimating the blood loss caused by hookworm (species not stated) in 3 patients. There was considerable variation in the blood loss per worm. The daily loss of 10-20 ml apparently accounted for the lowered hemoglobin values and the authors were of the opinion that toxins were not involved. However, the individuals had relatively light worm burdens and the authors stated that this and other factors might have counteracted the full effect of hookworm toxins.

Roche *et al.* (29) estimated blood loss in hookworm infection in 21 patients by tagging circulating erythrocytes with radioactive chromium. Blood loss per day in the stool was found to range from 2.0 ml to 251.5 ml and was in rough proportion to the severity of infection. In pure *Necator americanus* infections blood loss per day per worm was on the average $3.11 \times 10^{-2} \pm 1.73 \times 10^{-2}$ per millilitre. Blood loss per *Ancylostoma duodenale* was estimated to lie in the neighborhood of 0.2 ml per worm per day.

THE LARVAL NEMATODIASES

One of the outstanding developments in the helminthic diseases during the past several years is concerned with the pathology produced by larval nematodes. In the United States, zoonotic larval ascariasis or visceral larva migrans is being recognized with increasing frequency. Perlingiero and György (30), Zuelzer and Apt (31), Mercer *et al.* (32), and Behrer (33) were the first to describe these cases, which have occurred mainly in young children. Most of these authors associated the condition with the larvae of *Ascaris lumbricoides*. However, Beaver and associates (34) were of the opinion that the 3 cases observed by them were due to the larvae of *Toxocara canis*. Since that time, the larvae of the cat ascarid, *Toxocara cati*, have also been incriminated. While the dog and cat ascarids seldom develop to maturity in the human host, the larvae which migrate following ingestion and hatching of the ova are capable of causing serious pathological conditions in the human host. The potential exposure to these

parasites must be considerable in my own country, for instance, since it has been estimated that there are in the United States some 22 million pet dogs and 11 million domestic cats, the majority of which at one time or another probably become infected with *Toxocara*. Beaver (35) has recently reviewed the problem of visceral larval migrans.

Previous to the knowledge of zoonotic larval ascariasis, Wilder (36) had reported 46 cases in the United States in which nematode larvae were found in 24 enucleated eyes and a characteristic reaction in the other 22. While the larvae were tentatively identified as those of *Necator americanus*, it is possible that those cases were due to the invasion of the structures of the eye by *Toxocara* larvae.

Innes and Shoho (37) have reported on cerebrospinal nematodiasis, a neuroparalysis of sheep, goats, and horses in Japan and Korea and in goats in Ceylon. This condition occurs seasonally and has been known for many years, but its etiology was obscure until Japanese workers demonstrated that it was due to the invasion of the central nervous system by the larvae of *Setaria digitata*, a parasite of cattle, which is transmitted by various species of mosquitoes. The lesion of the central nervous system in the abnormal hosts consists of a focal malacia, with or without hemorrhage, which proceeds to liquefaction and cavitation, and results in acute or subacute clinical paralysis. Mohiyuddeen (38) has described subacute and chronic forms of enzootic bovine paraplegia in Mysore State of India. In acute cases, sudden and complete paralysis is followed by death in 2 to 3 days. Sheathed microfilariae have been isolated in the sediment from the spinal cord and brain. In 2 acute cases, immature nematodes, other than microfilariae, were present in the lumbar portion of the spinal cord. Innes *et al.* have suggested that identical human disorders may occur but have gone unrecognized. In this connection, Browne (39) has reported a case of nematodiasis of the central nervous system in a patient in the Belgian Congo believed to have been due to *Loa loa*. These demonstrated pathological conditions due to wandering larvae of nematode parasites lead one to suspect that aberrant larval nematodiasis may be of greater medical importance than heretofore conceived.

Since the classic discovery of Shope (40) that the swine lungworms, *Metastrongylus apri* and *M. pudendotectus*, carry the virus of swine influenza, evidence is accumulating that larval nematodes may

be involved in the carriage of other viruses. Syverton, McCoy, and Koomen (41) found that the larvae of *Trichinella spiralis* are capable of transporting the virus of lymphocytic choriomeningitis and Mochizuki, Tomimura, and Oka (42) have shown that *Toxocara canis* larvae can open the blood-brain barrier and pave the way for the localization of the virus of Japanese *B. encephalitis*. Jeffery (43) has recently demonstrated that *Nippostrongylus muris* can acquire St. Louis encephalitis virus from an infected host and that there is considerable multiplication of the virus in the free-living stages of the parasite. The carriage of viruses by helminth parasites is a potentially important field which needs further exploitation.

TRICHINOSIS

Some advances have been made in this field in recent years. Ker-shaw *et al.* (44) provided information concerning the distribution of the larvae of *T. spiralis* in the voluntary muscles of 2 patients coming to autopsy, as well as in biopsied muscle from 16 other patients. It was shown that in the case of a light infection the concentration was highest in the muscles of the forearm and the calf, and that in the heavily infected case it was highest in the tongue and diaphragm, although almost as high in the forearm and the calf. The severity of the illness and the intensity of infection were shown to be related, the more severe infections being associated with high concentrations of larvae and the milder ones with lower concentrations. In my own laboratory, we have accumulated similar data over a period of years on scores of cases of clinical trichinosis, but have not felt that valid conclusions could be derived from such data. On the other hand, there is a fair amount of evidence which would indicate that infections of 1,000 larvae per gram of diaphragm muscle are invariably fatal. Wright *et al.* (45) found that in 855 positive diaphragms of 5,313 examined from individuals coming to necropsy in 189 hospitals in 114 cities in 37 States and the District of Columbia, none had an infection of over 1,000 larvae per gram. Gould (46) failed to find any infections of this degree in the 278 positive cases in the 1,231 examined by him at Eloise, Michigan. In fact, Wright *et al.* (45)

stated that they were unable to locate in the literature any findings of 1,000 larvae or more per gram of diaphragm muscle in persons coming to necropsy from conditions other than clinical trichinosis. They concluded that «it appears probable that infections of this degree usually, if not invariably, result in death».

There appears to be no advantage in recording our numerous data concerning numbers of trichina larvae in biopsied muscle, but it may

*Numbers of larvae of Trichinella spiralis encountered in various muscles of individuals dying of trichinosis*¹

Case number	Age of patient (years)	Approximate number of days between infection and death	Method of examination ²	Average number of larvae per gram of muscle				
				Diaphragm	Gastrocnemius	Rectus abdominus	Psoas	Deltoid
1	?	54	D	2,677	—	—	—	—
2	57	21	MD	8	3	3	2	—
3	?	25	MD	553	—	453	603	—
4	64	24	MD	21.7	6.7	—	—	2.9
5	?	29	MD	77.6	171.3	—	—	—
6	52	33	MD	82	—	—	59	—
7	32	30	MD	1,230	—	—	250	—
8	22	25	MD	395	—	—	1,813	1,963
9 *	29	14	MD	175	—	—	—	—
10	13	25	MD	92.3	—	—	—	—
11	?	30	MD	583	—	—	—	—
12	50	20	MD	46	4	—	—	—
13 **	29	29	MD	39	4	—	—	18

¹ All examinations conducted in the Laboratory of Tropical Diseases, Bethesda, Md.

² D indicates examination of digested muscle; MD indicates direct microscopic examination and examination of digested muscle.

* In addition, the tongue contained 6 larvae per gram and the intercostal muscle 2 larvae per gram.

** In addition, the following muscles contained larvae per gram as indicated: right extraocular 1, left extraocular 2, masseter 9, right intercostal 16, right quadriceps femoris 20.

be of value to indicate (Table) the records of larval counts in individuals dying of the disease. The important factor to be noted in this table is the extreme limits of variability in the average number of larvae recovered from the various muscles. This may be accounted for in part by the time of death following exposure to infection. Since female *T. spiralis* probably continue to produce living young for

6 weeks or longer, the maximum concentration of larvae in the voluntary muscles will not have been reached before that time. In fact, in most, if not all, of the 13 cases recorded in the Table, second stage larvae were encountered and in some of them first stage larvae were also seen. With the marked variations noted in larval counts in persons dying of trichinosis, it would be difficult indeed to draw conclusions concerning the relationship of larval concentrations in biopsied muscle and the severity of the symptoms.

Gould *et al.* (47) showed that a dose of 750,000 r. filtered X-ray applied to trichina larvae *in vitro* caused death of all or nearly all larvae within 1 and 2 hours after irradiation. Reproduction of trichinae was largely prevented by exposing larvae *in vitro* to X-radiation of 3,500 r.; maturation to adult worms was largely prevented by exposure to 5,000 or 6,000 r. Pieces of trichinous meat in thicknesses of 1.45 cm were rendered non-infective to experimental rats when the meat was exposed to 15,000 r. The authors suggested that the dose of X-radiation required to sterilize trichina larvae in commercial quantities of pork may not be excessive.

Gould *et al.* (48) employing cobalt⁶⁰ believed that a dose of 30,000 r. would prevent the maturation of trichina larvae. No changes in the flavor or texture of pork were produced by doses of approximately 40,000 r. with Co⁶⁰. The authors concluded that the exposure of all raw pork to gamma radiation of waste fission products may be a practical means of controlling trichinosis in man and swine.

In the United States where Wright *et al.* (45) reported that 16.4 percent of 5,313 persons coming to autopsy were infected with *T. spiralis*, progress has been made in the control of the disease in a completely incidental fashion. The widespread distribution of vesicular stomatitis in swine has led during recent years to the promulgation of regulations and laws requiring the cooking of raw garbage prior to its consumption by swine. Such regulations or laws are now in force in 47 of the 48 States. No doubt these measures will result in reducing materially the exposure of the human population to trichina infection.

GNATHOSTOMIASIS

The recognition of gnathostomiasis as a medical problem in Japan after World War II led to studies of the disease by many Japanese investigators. Miyazaki (49) has summarized much of this work. The infection is particularly prevalent in Kyushu and Shikoku, but occurs also in Honshu. There are three species of *Gnathostoma* in Japan, viz. *G. spinigerum*, *doloresi*, and *nipponicum*, the first of which is most important medically. The natural hosts of *G. spinigerum* are dogs and cats. Of the former 26, or 4 percent, of 643 examined in Kyushu were positive, as were 13, or 35 percent, of 37 cats. Four species of *Cyclops* constitute the first intermediate hosts, viz. *Mesocyclops leuckarti*, *Eucyclops serrulatus*, *Cyclops strenuus*, and *C. vicinus*. The second intermediate hosts become infected either through consumption of the primary hosts or through other secondary intermediary hosts. In the natural world, 6 species of fresh water fish, 2 of frogs, 3 of snakes, 16 of birds, and one mammal have been newly recorded as the secondary intermediate hosts of the nematode. Two species of fish, *Ophicephalus argus* and *O. tadianus*, are important intermediate hosts. An intradermal test with a 1:50,000 dilution of antigen prepared from the imagines or larvae has been found of value for diagnosis of infection.

CESTODE DISEASES

Marked progress has been made in our knowledge of the cestode diseases since the last Congresses. The long standing riddle of alveolar echinococcus has been solved through the studies which have been summarized by Rausch (50), Rausch and Schiller (51), and Vogel (52). Rausch and Schiller reported *Echinococcus multilocularis* Leuckart, 1863 (syn. *E. sibiricensis* Rausch and Schiller, 1954) to be the cause of alveolar echinococcus in man on St. Lawrence Island, Alaska, and showed that microtine rodents, particularly *Microtus* spp. and *Clethrionomys rutilus*, are the natural intermediate hosts of this cestode, although other species of mammals, including man, are parasitized with varying degrees of success on the part of the larval cestode. The arctic fox is the natural final host of the parasite on St. Lawrence

Island. Rausch (50) reported the occurrence of *E. multilocularis* on the mainland of Alaska.

In Europe, Vogel (52) studied the epidemiology of *E. multilocularis* in the Swabian Alps and found that the life cycle was essentially that encountered by Rausch and Schiller on St. Lawrence Island. Vogel identified worms from a dog fed human alveolar material by Posselt (53) in 1901 as *E. multilocularis*, thus providing complete proof of the life cycle, in which, in contradistinction to *E. granulosus*, herbivora play no role. A detailed review of the studies on *E. multilocularis* will be presented on this program.

Gradually the mechanism of the production of pernicious anemia by *Diphyllobothrium latum* begins to unfold. Von Bonsdorff (54) has shown that *D. latum* contains considerable amounts of vitamin B₁₂, and that dried tapeworm material may be employed as «extrinsic factor» in Castle's test. Aqueous extracts of the tapeworm injected parenterally bring about maximal hematologic remission and improvement of the neurologic disturbances. Nyberg (55) employed Co⁶⁰ labelled vitamin B₁₂ in measuring the activity of the vitamin by *D. latum* and found that such was remarkably constant, indicating that the vitamin is an essential constituent of the tapeworm. The same author (56) showed that *D. latum* absorbs an average of about 44 percent of a single oral dose of radioactive vitamin B₁₂ given to the host. The individual variations are great and seem to be due to a variety of unpredictable factors. *Taenia saginata* failed to take up any radioactive vitamin B₁₂ when the material was administered to the host in a single dose by mouth. Previously Nyberg (57) showed by biological methods that extracts of *D. latum* contained vitamin B₁₂ activity corresponding, on the average, to 2.3 µg per gram of dried tapeworm, whereas *Taenia saginata* had a corresponding value of only 0.046 µg. Other factors concerned in the mechanism have not been entirely elucidated. However, we are having on this program the latest authoritative information on this subject.

ANTHELMINTIC MEDICATION

Since the introduction by Mouriquand *et al.* (58) in 1951 of piperazine hydrate in the treatment of *Enterobius vermicularis* infec-

tions, various salts of piperazine have been employed successfully against both pinworms and *Ascaris lumbricoides*. Some success has been achieved in the treatment of *Opisthorchis* (59) and *Clonorchis* (60) infections with large doses of chloroquine diphosphate and, on the basis of limited evidence, it would appear that *Paragonimus* infections may be partially controlled by this therapy (61). The corticoid steroids have given relief for many of the symptoms in trichinosis, although they apparently have no effect on the adult or larval worms. Finally, Dithiazanine (62) has been found effective for *Trichuris trichiura* and *Strongyloides stercoralis*, for which adequate treatments have heretofore not been available.

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VITAMIN B₁₂ DEFICIENCY IN CARRIERS OF *DIPHYLLOBOTHRIUM LATUM*

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Vitamin B₁₂ is of essential importance to the human body. Its presence is necessary for the biological synthesis of nucleoproteins and, thus, for the maturation process of the cell nuclei.

B₁₂ deficiency in man manifests itself in the form of a well defined picture of disease, identical with what has long been known as Addisonian or genuine pernicious anaemia. This deficiency state is relieved by adequate B₁₂ therapy.

B₁₂ is absorbed from the intestinal canal only in the presence of Castle's «intrinsic factor», which is secreted in the stomach. Its nature is as yet not fully understood, however. Normally, a considerable proportion of the B₁₂ ingested with the food is absorbed, transferred to the blood and deposited in the B₁₂ pool. The main constituent of this is the B₁₂ stored in the liver. There is also an enterohepatic circulation of B₁₂ — Okuda, Gräsbeck & Chow (16); Gräsbeck, Nyberg & Reizenstein (9) —, an appreciable amount of this substance being excreted with the bile and subsequently reabsorbed. Furthermore, a synthesis of B₁₂ occurs in the caecum and the colon, but this product cannot be utilized by the human body itself.

A B₁₂ deficiency develops when the supply of this substance is interrupted for so long that the B₁₂ pool is more or less completely exhausted and the B₁₂ concentration in the serum drops below the critical level (about 100 µµg per millilitre). This may occur if B₁₂ is lacking in the diet for a very long period of time. It also occurs if intrinsic factor is lacking, as is the case after total gastrectomy and in atrophy of the gastric mucosa, i.e. in genuine pernicious anaemia.

Intestinal disturbances may cause the development of an abnormal bacterial flora consuming B_{12} . And, finally, B_{12} deficiency may be caused by the fish tapeworm, *Diphyllobothrium latum*.

It has been known for 70 years that the fish tapeworm sometimes causes a picture of disease, in all respects corresponding to that of genuine pernicious anaemia (1, 2, 3, 4). Since infection with the fish tapeworm is commoner in Finland than anywhere else, it is only natural that this disease has been mainly studied by Finnish investigators. At the time when genuine pernicious anaemia was still an incurable disease, i.e., before liver therapy was introduced (1926), it had already been observed that tapeworm anaemia could be cured by expulsion of the worm. Otherwise, the only clinical difference between tapeworm anaemia and genuine pernicious anaemia is that young people, too, may develop the former (the youngest recognized victim was 9 years old), and that the gastric juice of these patients contains intrinsic factor.

All these facts were known 20 years ago, when I began my investigations. By contrast, the pathogenesis of tapeworm anaemia was not understood. The view most commonly held was that the disease was due to poisons produced by the worm and absorbed by the host. My personal starting-point was the hypothesis that the essential fact was the presence of the worm in the intestine. Castle's observations had already revealed the essential significance of the alimentary tract for the development of the factors protecting against pernicious anaemia.

After ten years' clinical experiments I was able to postulate that the old toxin theory was obsolete (2). My investigations had shown that in tapeworm anaemia the stores in the body of «liver factor», or «antianaemic substance», to use the terminology of the time, were exhausted. The obvious cause seemed to be that the living worm in the intestine in one way or another interfered with the interaction between «intrinsic factor» and the «extrinsic factor» supplied with the food (which we now know is identical with B_{12}). Mixtures of normal gastric juice and extrinsic factor-containing food stuffs (meat, in the first place) produced no remission in patients with tapeworm anaemia, in contrast to what is the case in genuine pernicious anaemia. I was also able to show that in tapeworm carriers with anaemia the worm was situated markedly higher up in the intestine

than in non-anaemic worm carriers. When the worm was forced to a more distal site without being removed, remission of the anaemia resulted.

The year 1948 brought with it the detection of vitamin B_{12} . It appeared that my results were readily compatible with the consequent new outlook with regard to pernicious anaemia. My investigations were continued in co-operation with several other workers. Our main object was to disclose in which way the worm disturbed the interaction between intrinsic factor and extrinsic factor, or B_{12} . We surmised that the tapeworm absorbed and somehow utilized the antianaemic substance. Very soon we were able to show — v. Bonsdorff & Gordin (5, 6) — that expelled tapeworm successfully could be used as a source of extrinsic factor in Castle's test, and that extract of dried tapeworm, parenterally injected, had a strong antianaemic effect. Simultaneously, it was demonstrated by microbiological methods — Nyberg (10) — that the worm substance had a very high B_{12} activity.

Expressed in terms of B_{12} , the situation was then as follows: We had conclusively shown that tapeworm anaemia is a manifest B_{12} deficiency with exhaustion of the B_{12} pool, and we had found that the tapeworm contained large amounts of B_{12} . Hence we drew the obvious conclusion that tapeworm anaemia is due to the worm absorbing B_{12} in competition with the host, thus sometimes, with the course of time, depriving the latter of his reserves of B_{12} .

One or two years later, radioactive B_{12} preparations became available and could be used in the study of the B_{12} metabolism. The tracer substance mostly used is Co^{60} -labelled B_{12} . Using the isotope technique, the metabolism of B_{12} can be studied with greater accuracy than what is possible in regard to many other substances, notwithstanding the minute quantities involved. The methods employed are the faecal excretion test, determination of the uptake in the liver (Glass' test), the urinary excretion test (Schilling's test) and determination of absorbed radioactive B_{12} in the serum. The last-mentioned method has not yet attained any practical significance, however.

During the last few years it has been chief concern of my co-workers to study the B_{12} metabolism in worm carriers using the methods just mentioned. The object of these investigations has been in part to determine the uptake of B_{12} in the host, in part to determine the uptake in the worm.

1) By the faecal excretion test the proportion of an orally administered, known amount of B_{12}^* excreted with the faeces is determined. It may be assumed that the rest has been absorbed. Normally, about 80 percent of a B_{12} dose of the magnitude 1 μg are absorbed. In genuine pernicious anaemia, where intrinsic factor is lacking, less than 10 percent are absorbed, the rest being recovered in the faeces. If, in such cases, the B_{12} dose is given together with intrinsic factor (normal gastric juice), absorption is normal.

In infection with *D. latum*, the situation is more complicated. A proportion of B_{12}^* is absorbed, another proportion is taken up by the tapeworm, and the rest is excreted with the faeces. In order to determine the absorption, it is necessary not only to analyse the faeces, but also the concentration of B_{12}^* in the tapeworm, which has to be expelled immediately after the test. It appears — Nyberg (11) — that the uptake by the tapeworm and, consequently, the absorption show a wide variation depending on the amount of B_{12}^* administered, but probably also depending on the amount of tapeworm present in the intestine, the location of the worm in the latter, the degree of activity of the parasite, etc.

In manifest tapeworm anaemia most of the B_{12}^* administered is found in the tapeworm, if the dose is «physiological», i.e., of the magnitude 1-3 μg . A smaller proportion is recovered in the faeces. In these cases absorption is ≈ 0 . Absorption is, however, markedly decreased — by about 55 percent, on average — in non-anaemic worm carriers, too (Fig. 1).

2) The uptake in the liver (Glass' test) has been determined in only one case of tapeworm anaemia by a team of Swedish investigators — Brante & Ernberg (8). In normal cases the surface radioactivity, determined with a scintillation counter, is concentrated over the liver during the first few days following oral administration of B_{12}^* . In the case just referred to the test showed that the activity over the liver was insignificant. The highest concentration was observed to the left of the abdomen, at a site where the patient occasionally felt pain (Fig. 2). It could be surmised that the tapeworm was situated in this part of the abdomen and that it had absorbed all of the B_{12} dose that had not been excreted with the faeces.

3) The urinary excretion test (Schilling's test) has attained a wide application in the study of the B_{12} metabolism. The patient is

given a dose of 0.5-2 μg B_{12}^* by mouth. One and a half hours later a «flush dose» of 1 mg non-labelled B_{12} is injected intramuscularly. The urine is collected for 24 hours. Where the absorption of B_{12}^* is normal, 10 percent, at least, of the dose administered is usually found in the urine. In genuine pernicious anaemia the yield is 0-2.5 percent. If, in this disease, intrinsic factor is administered together with the B_{12}^* dose, values within the normal range are obtained.

In five cases of tapeworm anaemia — Nyberg, Gräsbeck & Sippola (14) — the excretion values were of the same magnitude as in genuine pernicious anaemia. About a week after expulsion of the

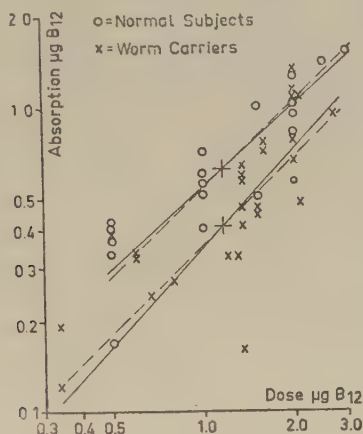


Fig. 1 — Faecal excretion test. The absorption of radioactive B_{12} in normal subjects and in non-anaemic carriers of *D. latum* following oral administration — Nyberg (11)

worm, normal values were obtained in the same patients. It was found, however, that worm carriers without anaemia also had a definitely decreased excretion, which increased significantly after the worm cure (Fig. 3). Like the results of the faecal excretion test, this seems to suggest that B_{12} absorption is inhibited in non-anaemic worm carriers, also.

The uptake in the tapeworm cannot be satisfactorily studied as long as it is impossible to keep the worm alive outside the human body. In connection with the faecal excretion test it was already mentioned that a proportion of B_{12}^* is found in the expelled worm. It is not

evenly distributed in the worm (Fig. 4). The proximal parts of the parasite have a greater avidity for orally administered B_{12} (calculated

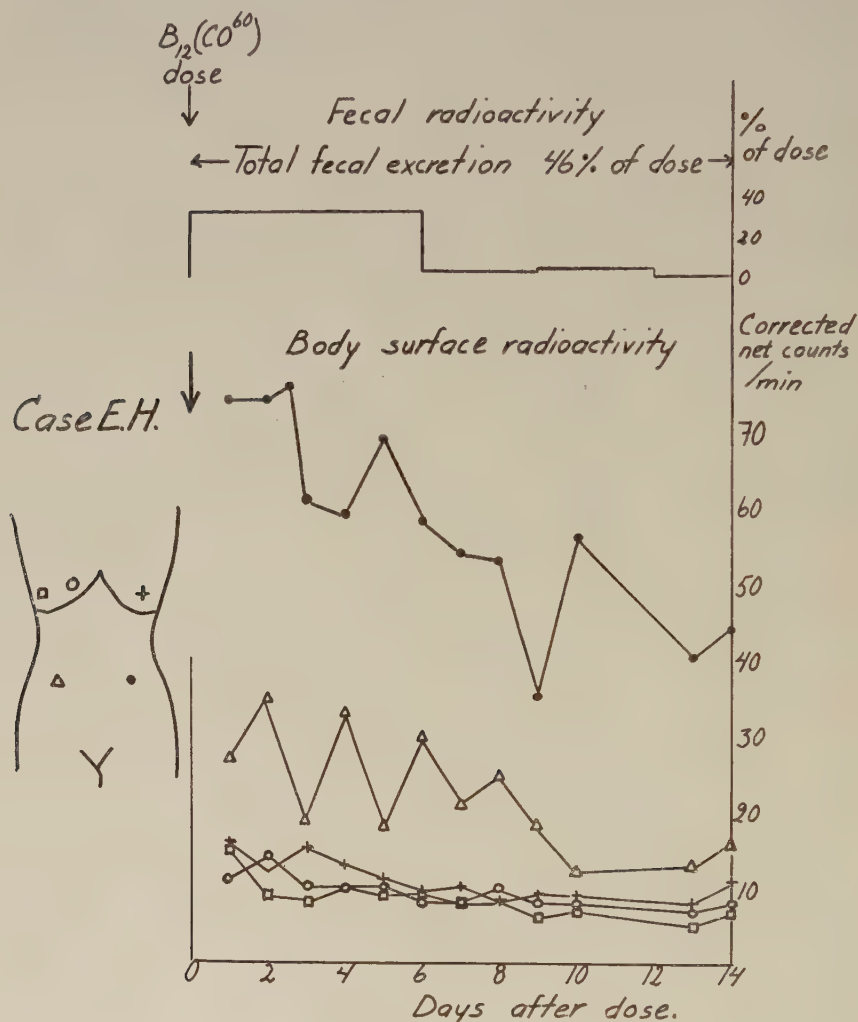


Fig. 2 — Glass-test in a case of pernicious tapeworm anaemia — Brante & Ernberg (8)

per gram of dry substance) than the distal parts — Nyberg (19). Considering that the growth of the worm mainly occurs in the proximal proglottides, it may, perhaps, be concluded that B_{12} is of essen-

tial importance to the worm from this standpoint. Radioactive B_{12} can also be visualized in autoradiograms of expelled worm (Nyberg; Brante & Ernberg),

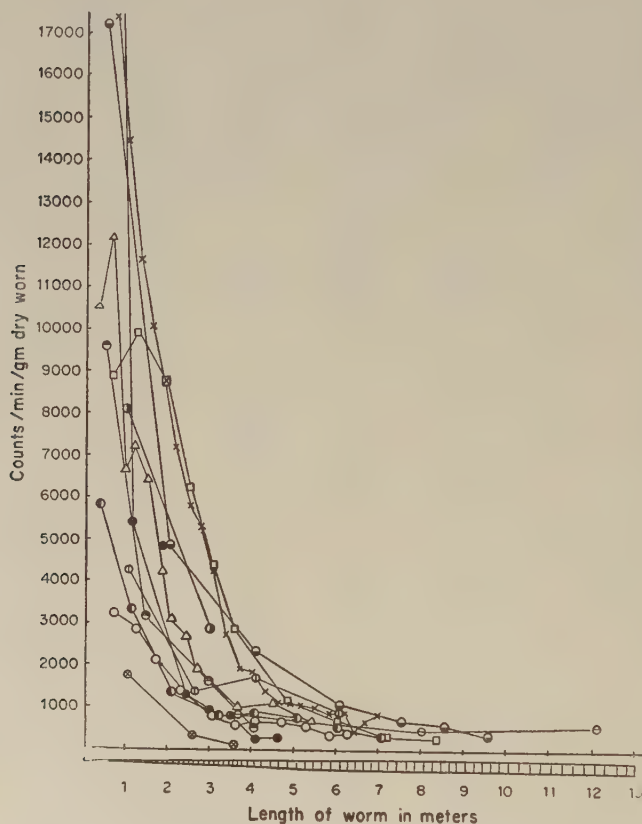


Fig. 4 — Distribution of radioactive B_{12} -uptake in counts/minute/g of dry worm piece of worm in 12 specimens of *D. latum* — Nyberg (12)

In the tapeworm, B_{12} occurs in a non-dialyzable form, probably bound to a protein — Nyberg & Gräsbeck (13). It is released, for instance, by autolysis and proteolytic digestion.

The uptake of B_{12} in the worm can also be studied *in vitro* by placing pieces of recently expelled tapeworm in a medium containing B_{12}^* — Brante & Ernberg (7); Nyberg. Investigations in process are

concerned with the mechanism of this uptake and with the question of whether the presence of intrinsic factor in the medium influences the uptake of B_{12} , etc.

All the results hitherto obtained corroborate the assumption that the uptake of B_{12} is metabolic and of essential significance to the tapeworm in some way or other. This property appears to be specific for *D. latum*. To the best of my knowledge, B_{12} deficiency does not occur in infection with any other parasite, not even with closely related species of *Diphyllobothrium*. Our team has been in a position to make comparisons only with *Taenia saginata*. It was found that this cestode does not absorb B_{12}^* , and that its content of B_{12} , microbiologically determined, is only about 2 percent of that of *D. latum* — Nyberg (10).

The results of the investigations with radioactive B_{12} have confirmed our previous observations and in many respects broadened our outlook with regard to the problems involved. Furthermore, they have convincingly disclosed the important fact that B_{12} absorption is disturbed in practically all carriers of the fish tapeworm, irrespective of whether a manifest B_{12} deficiency has developed. With regard to the B_{12} metabolism no sharp distinction can thus be made between anaemic and non-anaemic worm carriers. It has to be taken into account that infection with *D. latum* will always more or less jeopardize the B_{12} standard of the host, a fact hitherto not recognized. This is of essential significance from the standpoint of public health in a country like Finland, where it is roughly estimated that about 20 percent of the 4.3 million inhabitants harbour the fish tapeworm.

The relatively low incidence of manifest B_{12} deficiency is mainly due to the fact that the B_{12} pool in the body is large. It takes a long time before it is exhausted. Probably the competition between the host and the parasite for the desirable B_{12} oscillates during the course of the years, periods with better absorption alternating with periods when absorption is poor. From our investigations we have concluded that absorption is inadequate especially when the worm is situated high up in the intestine, when the amount of worm present is very large, when the supply of B_{12} in the food is inadequate, or if the secretion of intrinsic factor is decreased. It is also probable that the need of the host for B_{12} varies, being particularly great in connection with pregnancy, febrile diseases, thyrotoxicosis, etc.

At present we are investigating the situation with regard to B₁₂ in a population heavily infected with the fish tapeworm. With financial aid from the National Institutes of Health, U.S.A., a field investigation in eastern Finland was undertaken last spring. The leader of the expedition was Dr. Nyberg. It is not possible to determine the B₁₂ pool by any method now available, but it may be assumed that the B₁₂ concentration in the serum is a fairly good index of the B₁₂ standard of the subjects investigated. Within a limited geographical district a non-selected group was studied. The investigation included a physical examination, examination for tapeworm ova, determination of the erythrocytes, the haemoglobin and the haematocrit value, as well as the collection of serum for B₁₂ analysis. The results of the last-mentioned studies are not yet available. A total of 1,344 subjects was investigated, tapeworm ova being detected in 366 (27.2 percent). Among the worm carriers there were as many as 8 with manifest pernicious anaemia. This corresponds to an incidence of about 1:50, which is higher than the figures hitherto presented, but it should be borne in mind that systematic haematological studies have never before been carried out among the population in any region notorious for tapeworm infection. Only a minute proportion of all tapeworm carriers in the country, or even of those on whom a worm cure has been performed, has been investigated by experts. It will take some time yet until the preparation of our material is finished.

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THE SIGNIFICANCE OF ECHINOCOCCOSIS IN THE AMERICAS

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Echinococcosis or hydatidosis is one of the more important zoonoses and it represents a serious threat to human health in several parts of the world. In the Western Hemisphere it has been recognized in boreal North America and in the southern countries of South America. In the tropical areas it occurs sporadically. There are some biological and epidemiological variations in both main foci of *Echinococcus* in the Americas and we shall intent to point out some of the most important differences.

ECHINOCOCCOSIS IN NORTH AMERICA

Up to now, two species of *Echinococcus* have been proved to occur: *E. granulosus* (Batsch, 1786) and *E. multilocularis* Leuckart (1863). Sylvatic cycles of both tapeworms have been described (Rausch and Schiller, 1951; Thomas, 1954; Riley, 1933, 1939; and Magath, 1954).

The life cycle of *Echinococcus* takes place among certain carnivora and different wild herbivora or small rodents.

Echinococcus multilocularis

The occurrence of the larval stage of *Echinococcus* species in a microtine rodent, *Clethrionomys rutilus* Pallas, on Behring Island was reported in 1938 by Barabasch-Nikiforov and in 1941 by Afa-

nas'ev (quoted by Rausch, 1956). In 1951, Rausch and Schiller found the cestode in tundra voles, *Microtus oeconomus* Pallas, captured on St. Lawrence Island, and afterwards they found it in *C. rutilus*. Experimental infections of various mammals proved beyond doubt that the species of *Echinococcus* found was *E. multilocularis* Leuckart, 1863. As Thomas and Babero (1956) and Vogel (1955, 1957) have pointed out, the name *E. sibiricencis* n. sp. Rausch and Schiller (1954a) has become a synonym. The adult tapeworm was found in foxes (*Alopex* and *Vulpes*) and, according to Rausch and Schiller (1956), was probably brought from Asia by animals carried by the pack ice. The incidence of infection in the arctic fox ranged from 40 percent in the winter of 1950-1 to 100 percent in the winter of 1954-5. The adult cestode has also been found in dogs on St. Lawrence Island, but its incidence in them is relatively lower (12 percent) (Rausch and Schiller, 1956). *E. multilocularis* has also been reported from continental Alaska by Rausch (1956a) and it was collected from foxes at four localities in arctic Alaska and from Nunivak island, too.

«The controversy of the past regarding the identity of the cestode causing alveolar hydatid disease is now clarified by the recognition of the coexistence of two species, *E. granulosus* and *E. multilocularis*, which differ in host-relationships and morphological characteristics. These species, however, are known to exist together only in boreal regions, the distribution of *E. multilocularis* being restricted by the occurrence of suitable intermediate hosts (rodents of the genera *Microtus*, *Clethrionomys* and *Lemmus*).

Although *E. multilocularis* is known only from parts of Alaska having an abundant mammalian fauna, it is believed that conditions in southern Canada and the United States are favorable enough to permit its becoming established, should it be introduced via infected dogs from the north.» (Rausch, 1956a.)

The occurrence of alveolar hydatid disease in man at St. Lawrence Island has been studied by Rausch and Schiller (1956) particularly with the use of the Casoni and complement-fixation tests. Unfortunately they only evaluated the immediate observations (20 minutes after the intracutaneous injection of 1/100 ml of antigen) of the Casoni tests and omitted the delayed type of reactions, which, according to our own experience, are more specific and dependable.

Rausch and Schiller (1956) have emphasized the epidemiological significance of their findings, having also outlined the prophylactic measures to be adopted for the control of alveolar hydatid disease.

E. granulosus

Adult cestodes have been found in different carnivora, mainly in canine animals. It does not seem capable to subsist in feline species, as Lörincz (1933) has proved. Unlike *E. granulosus*, *E. multilocularis* matures in cats and foxes. The occurrence of *E. granulosus* infection in wild carnivora in North America is of great significance and was first reported by Riley (1933) in 2 wolves (*Canis lupus*) and afterwards (1939) in 5 out of 12 timber wolves (*Canis lupus lycaon*) which were killed in the northeastern part of Minnesota. Miller (1953) stated that *E. granulosus* was well established in *Canis lupus* in northern Canada and to some extent in the more southern provinces of Canada. In all these areas, hydatid cysts have been found in the lungs of wild herbivora, particularly the moose (*Alces americana*) and the caribou (*Rangifer tarandi*). The maintenance of the sylvatic cycle of *E. granulosus* is assured when wolves devour the lungs infected with hydatid cysts of the wild herbivora.

Dogs have also been found infected. In the United States the infection appears to be rare and the surveys have been negative. Franklin and Ward (1953) reported, in Mississippi, 2 dogs over 50 with very heavy natural infections of *E. granulosus*. «However, recent reports in regions where hogs are known to be most frequently infected and where cases of indigenous hydatid disease have been observed suggest strongly that foci of infection in dogs may be slowly developing in the United States.» (Magath, 1954.)

In Canada, Miller (1953) has reported the infection of dogs. He examined 114 dogs and found 32 (25 percent) infected ones. More recently, Wolfgang has estimated the number of Indian dogs per village on the Mackenzie River at a minimum of 50 with a high of 2,000 at Aklavik. He examined 27 dogs and found 7 (25 percent) to be infected (Wolfgang and Poole, 1956). In the northwest territories of Canada and in British Columbia domestic herbivores are non-existent and the life cycle of *Echinococcus* is obviously dependent on sylvatic infections of wolves and wild herbivores.

According to Magath (1954), about 40 cases of autochthonous hydatidosis in man have been reported in the continental United States and more than 600 patients had acquired their disease in some foreign country. In Canada and Alaska, the epidemiology appears to be different. Extensive X-ray surveys for tuberculosis among the North American Indians and Eskimos have shown 98 autochthonous cases of pulmonary hydatidosis, 28 of which were confirmed by surgery or post-mortem examination (Miller, 1953). Sweatman (1952) thinks that human infections can be acquired «as a result of handling infected wolves but more probably from contact with dogs that had eaten infected carcasses of moose or more rarely some other wild herbivore. Dogs found in the vicinity of hunting lodges area in danger of becoming infected. If transported to farming districts in southern Canada, these animals could act as a source of infection for both humans and farm animals».

The risk of man to acquire hydatidosis is greater the closer the association they keep with their dogs. Surveys with Casoni tests were performed by different authors (Miller, 1953; Rausch and Schiller, 1956; and Wolfgang and Poole, 1956). Wolfgang and Poole tested 2,022 Indians in Canada's Northwest and obtained 31 percent of immediate positive reactions; but, according to our experience, the late lecture, made 16 to 24 hours after the injection of the antigen, would have given more specific results. It is probable that most of the positive results recorded by Wolfgang and Poole and some of the other authors previously mentioned might be unspecific.

Human cases have been diagnosed in Canada and Alaska in localities close to moose and wolf populations. The Indian Health Services Hospital, located at Edmonton, Alberta, has recorded 180 human cases of hydatidosis. In northwestern Canada the Indians use the infected viscera of wild herbivora to feed their dogs (Wolfgang and Poole, 1956).

ECHINOCOCCOSIS IN CENTRAL AND SOUTH AMERICA

Echinococcus granulosus has been reported in Guatemala (Aguilar, 1953), in Venezuela (Vogelsang and Herrera, 1949), in Colombia, Ecuador, Bolivia, Peru and Paraguay (V. Pérez Fontana, 1949).

But the highest prevalence of the infection occurs in Uruguay, Argentina, Chile, and in one state of Brazil: Rio Grande do Sul. According to the statistics published by different authors (A. Ferro, 1958; V. Pérez Fontana, 1949; and Neghme and Silva, 1956), we can approximately estimate that more than 20,000 human cases have been diagnosed in the last 10 years (Fig. 1). The infection of domestic herbivora also reaches high figures in these countries and, although there are no up-to-date statistics, in my opinion the infection rate among sheep, cattle, and pigs may vary from 10 to 30 percent.

Hydatidosis is prevalent wherever man, dogs, and sheep are closely associated. According to Dévé (1949), the geographical distribution of hydatid disease in man is parallel to that of sheep. The only exception to this statement occurs in the United States of America. Dévé thought that this was due to the good and systematic protection of the flocks of sheep against dogs. 90 percent of the hydatid cysts in sheep show scolices of *E. granulosus*, while the fertile cysts in cattle amount to 10 percent and in swine to 20 percent (Boero, 1950). The veterinary inspectors who control the meat in the slaughter houses condemn the infected viscera, but slaughtering without veterinary control is frequent in the rural areas and dogs have many opportunities to feed on lungs and livers infected with hydatid worms of sheep, cattle and pigs. Infection rates in dogs vary in Chile from 4.6 to 27 percent and they are the most important factor in the spread of infection among the intermediate hosts (Neghme, Rivera and Alvarez, 1956). The number of stray and superfluous dogs is high and they sow the eggs of the tapeworm on the soil, water, vegetables, and pastures in rural areas and suburban zones of towns. Eggs may also be mechanically conveyed by house and filth flies. The number of dogs in a locality and their opportunities of feeding on viscera of herbivores are important factors in the high rate of infection in man.

Sheep-raising and cattle-raising areas of Chile, Argentina and Uruguay are, of all American countries, the most heavily infected with hydatid disease. In Chile, every year occur at least 500 new human cases and during 1957 the infection was of 7.6 per 100,000 inhabitants. The disease in human beings has a chronic evolution and most of the infections are produced in childhood. This may be due to the close association of children with dogs. The symptoms in man vary widely



Fig. 1 — Distribution of human hydatidosis in some South American countries according to data of Perez Fontana (1949), Ferro (1957), and Neghme and Silva (1958)

according to the location and the size of the cyst. The largest number of cysts is found in the lungs (49.3 percent) and in the liver (36.5 percent) (Pilotti and Faiguenbaum, 1951). In Chile, in the periods from 1945 to 1954 and 1956 to 1957, we have recorded 4,528 human cases and a death rate of 6 percent (Neghme and Silva, 1956b).

In most instances, surgery is the only available treatment, except when the patient condition is poor. In this case biological treatment prior to surgery is indicated (Faiguenbaum and Fanta, 1953).

Sylvatic echinococcosis

In 1863, Diesing (quoted by Cameron, 1926) described in the South American jaguar (*Felis concolor*) a species of *Echinococcus* which he believed to be different from *E. granulosus* and which he called *Taenia oligarthra*; in 1926, Cameron (loc. cit.), examining a South American cat, *Felis yaguarundi*, which died at the London Zoological Gardens, found other tapeworms which appear to belong to the same species of taenia and which he called *E. oligarthrus* (Diesing, 1863). Both descriptions were based only on adult cestodes and no experimental infections were performed in order to study the larval stage. In Africa, Ortlepp (quoted by Rausch, 1953) has described *E. lycaontys* in *Lycaon pictus* and *E. felidis* in *Felis leo*. Up to the present neither *E. oligarthrus* nor *E. felidis* have been found in canine hosts and nothing is known regarding the mammals parasitized by the larval stage nor its characteristics. Recently, Yamashita, Ohbayashi and Konno (1956) have made an evaluation of the morphological characters to be used as the basis for species differentiation and for obtaining a more exact knowledge of the morphological changes during the development of *E. granulosus*. They have found that there are variations in the size and in the shape of the various organs not only at the same stage after infection but also along the development of the cestodes. Consequently, they have pointed out, «it is very necessary for the differentiation of *Echinococcus* spp. to understand such variations sufficiently and to carry out further work, on the basis of experimental infections of other species». Based on this work and on our experience, we believe that the validity of the different new species of *Echinococcus* sp., based on the description of a few or several

specimens of tapeworms alone, could be questionable and further experimental work is desirable to clarify their relationship. Thus far, the only valid species are *Echinococcus granulosus* and *E. multilocularis*. We accept the validity of the latter, after the experimental work performed by Vogel in Germany (1955, 1957), by Thomas Babero (1956a) and by Rausch and his associates (loc. cit.). *E. multilocularis* can be distinguished, besides of important morphological differences of the adult cestode, because its larval stage is found in small rodents (*Microtus* and *Clethrionomys*), displaying a characteristic alveolar structure.

Brumpt and Joyeux (1924), in examining a specimen of *Dasyprocta aguti* L. captured at a locality of the state of São Paulo, Brazil, observed a hydatid cyst. He believed this tapeworm to be a new species and described it as *E. cruzi*. Cameron (1926) thinks this cestode is *E. oligarthrus*. But, again, no experimental infection was performed. Infection with unilocular hydatid cysts has been found in Peru, in lungs and livers of *Lama glama* and *Lama glama pacos* (Arnao, 1951), which are domesticated in several zones of the Peruvian and Chilean Andes.

De la Barrera (1948) found the larval stage in Argentina, in 4 over 104 specimens *Microcavia australis* autopsied at Território de Rio Negro. Najera (1950) and Mayer, in examining a group of the cervidae, *Mazama simplicicornis* living in captivity and in contact with dogs in Argentina, found 7 cysts in the peritoneum and 8 in lungs of one specimen. Montero (1958), several years ago, found hydatid cysts in the liver and in the peritoneum of a «huemul» (*Hippocamelus bisulcus*, Molina), captured alive at the Andes Mountains, near Chillan, Chile.

Under the auspices of the Pan American Sanitary Bureau (Regional Agency of the World Health Organization), we have begun in January 1955 a survey of echinococcosis in wild mammals in different zones of Chile. Up to the present time (August, 1958), we have explored the territories ranging from 31° to 43° southern latitude. We have had the opportunity to examine 6,936 animals, of which 238 were *Dusicyon culpaeus* and 82 *Dusicyon griseus*. These 320 foxes were negative for *Echinococcus* sp. Three specimens of *Felis concolor* were also negative. Most of the other specimens captured



Fig. 2 — Hydatid cyst of the liver found in a captured specimen of *Octodon degus degus*, Molina

were small rodents. Out of a total of 3,171 specimens of an indigenous rodent, *Octodon degus degus*, Molina, examined, we have found one specimen infected with hydatid cysts (Figs. 2 and 3). The experimental



Fig. 3 — Same as Fig. 2, but with higher magnification. Several brood capsules can be clearly observed through the cyst wall

infection of 2 dogs provided us after 60 days with the adult cestode, which showed the morphological characteristics of *E. granulosus*. Due to the extremely scarcity of the «huemul», an animal found only in

the Chilean and Peruvian Andes, we have been unable to capture any specimen in order to further Montero's (1958) observation. Our survey is still under course (Alvarez, Tagle, and Neghme, 1958). But we are under the impression that the sylvatic cycle, if it occurs at all, is of little epidemiological significance in South America.

We agree with Cameron (1957) that «hydatid disease was originally a condition of wild animals, as is still the case in sylvatic hydatid in North America, and the occurrence of the disease in man is mainly the result of agricultural practices. Under conditions of domestication, dogs replace wild carnivores and domestic herbivores replace deer, rodents, or marsupials. Man is then exposed to infection and contracts it» (Cameron, 1957).

But in South America, the origin of hydatidosis appears to be different from that of North America. Although it is not possible to say for how long the disease has been occurring in South America, it is quite probable that it was brought over from Spain after the conquest by the Spanish colonizers. It must be assumed that *Echinococcus granulosus* was introduced in South American countries with the dogs or sheep by the first settlers in the sixteenth or seventeenth century. Then, as the number of dogs, sheep and cattle increased, hydatid disease began to appear; at the beginning it may have occurred only sporadically. Gay (1849) reported the presence of *Echinococcus* in cattle and in man. From 1850 on the increasing number of cattle, sheep and dogs combined with the lack of sanitation and poor personal hygiene favored the transmission of hydatid disease to human beings. The relative scarcity of wild carnivora, which only rarely prey on sheep and cattle, provides an explanation for its low or practically nihil role in the spread of the infection in natural conditions.

Perez Fontana (1949) has advanced the opinion that hydatidosis may have been introduced by the Icelandic, English, and Norwegian whalers, who settled on both banks of the La Plata River. They brought with them a large number of dogs, and many of them may have harboured *E. granulosus*. From the La Plata River the infection extended slowly into the mainland and to the other countries. Infection of wild animals seems to be only a consequence of that of domestic herbivores and dogs and appears to be at the beginning stages in our environment.

CONTROL OF HYDATID DISEASE

Ignorance of people and communities about the life cycle of echinococcosis is responsible for the high prevalence of the disease. While in the rural areas of South America people use to feed the dogs with raw infected viscera of sheep, cattle or pigs, in North America, Indians and Eskimos of Northwestern Canada (Wolfgang and Poole, 1956) allow the viscera of the wild herbivores to be eaten by their dogs. Another important factor in rural endemic areas is the large number of dogs kept in more or less close association with individuals, who, in general, live under bad conditions of sanitation and have poor habits of personal hygiene.

Promotion of health education is the best prophylactic measure to be applied and it should be directed to obtain a permanent control over those personal acts permitting the infection of dogs with *Echinococcus* and thus the subsequent appearance of hydatid disease in man and domestic herbivores. This education should be started at the grade schools and should be expanded to the home and the whole community.

Programs developing rural communities in a broad sense are also highly desirable. Improvement of sanitation at the slaughter houses is a must. Dogs should not be allowed on the premises of the slaughter houses and measures should be taken to prevent their feeding with raw infected viscera. Proper control of the slaughtering of herbivores at farms and homes should be emphasized. Dogs should be kept as free of intestinal parasites as possible. This can be achieved by offering owners of dogs free periodical treatments of the animals with suitable taeniocides (Arecoline hydrobromide, in dose of 4 mg per kilo of weight).

It is very important to overcome the sentimental ties of the communities towards dogs by rationally justifying the elimination of stray and superfluous dogs on the basis of the health protection of the individuals and also of the useful or pet dogs.

Health education should be extended to all social levels and must emphasize the obligation of the individuals involved in the prevention of *Echinococcus* infection of dogs.

The experiences suffered by echinococcosis patients should be utilized in such programs. This should be done during and after the hospitalization of the patients.

A well directed psychological propaganda intended to inform public opinion should enhance the possibilities of an adequate general control of the disease. A close association of physicians, veterinarians, teachers, and other leaders of the community — organized in committees at the local and national levels — is highly desirable in order to coordinate the measures to be adopted in a rational campaign against the zoonosis.

In some provinces of southern Chile, an educational campaign against hydatidosis was started in 1952. Based on this experience, Sotomayor (1953) has outlined a general program of health education on echinococcosis.

In Iceland, after nearly a century of application of preventive measures, hydatid disease has disappeared. Dungal (1957) has analyzed the measures adopted in breaking the life cycle of *Echinococcus*. He concludes: «But probably no one cause is attributable for the great change which has taken place. A combination of circumstances, some purposely enforced and others fortunately associating themselves to the prophylactic measures, have resulted in eradicating a dangerous disease which had thoroughly infested the population to such a degree that its existence was endangered.»

SUMMARY

Echinococcosis or hydatidosis occurs in boreal North America and in the southern countries of South America. In so far as the tropical areas are concerned it has been reported sporadically.

The author analyzes the biological and epidemiological variations in the foci of *Echinococcus* in the Americas.

In North America, *Echinococcus granulosus* has been described in Alaska, Canada and United States in *Canis lupus*; the larval stage in moose and caribou. It has a sylvatic cycle and human cases occur sporadically. Dogs have also been found infected and in some places of Northwestern Canada they are fed with infected viscera of wild herbivora.

In South America, the most important enzootic and epidemiological foci occur in the sheep-raising and cattle-raising areas of Argentina, Uruguay, Southern Brazil and Chile. Hydatidosis reaches its highest incidence at the La Plata River mouth,

although some important enzootic and endemic foci also exist in Central and Southern Chile. The cycle of hydatidosis here is mainly the domestic one and sylvatic echinococcosis appears to be at the beginning stages.

Echinococcus multilocularis, which has a sylvatic cycle, so far has been found and demonstrated only in St. Lawrence Island and mainland Alaska.

The author analyzes the various biological and epidemiological factors of echinococcosis in the Americas. Based on this analysis, a rational program for the control of the disease is proposed. Promotion of health education is the best prophylactic measure to be applied.

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CONTRIBUTION TO THE STUDY OF INTESTINAL PARASITOSIS IN GOA—SOME CLINICAL AND LABORATORIAL DATA

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In a previous work of ours (1) we drew attention to the important problem of intestinal parasitosis as a factor of digestive carency. The fact is that there are cases in which clinical experience reveals the existence of signs of deficiency in certain individuals whose diets are not deficient, as if the loss of *minimum substances* took place after feeding. This can be seen in cases of digestive upsets, apparent or disguised at the level of the stomach and chiefly of the intestines. It is what Mouriquand calls *digestive carency*.

On the other hand, our daily observations have shown that, in tropical countries, there is an intimate connection between intestinal parasitosis, specially of roundworms infection, ankylostomiasis and amoebiasis, and condition of hypo- and avitaminosis. This association is so intricate that, in cases of hypo-proteinaemia, anemia, sprue, polynevritis of the beri-beri type or serous cachexia, it becomes difficult to fix the part played by each of those factors.

If in our previous published works we have demonstrated the importance of the nutritional factor, in this brief study (which is no more than an introduction, for a larger and a systematised investigation is imperative) we wish to dwell on the *factor of intestinal parasitosis*.

LABORATORY DATA

The first case of ankylostomiasis in Goa was diagnosed and confirmed by post-mortem, in 1906, by Wolfango da Silva. But there were no laboratory findings. The earliest laboratorial investigations were carried out in 1912, following the discovery of ankylostomiasis in Ribandar by Froilano de Melo (2). A little later, it was found that there was wide incidence of ankylostomiasis, due chiefly to *Necator americanus*, much more widespread that seemed at first sight, and to the frequency of infestation by *Ascaris* and *Tricocefalus*. In 1925, Humberto Rodrigues (3), working under Froilano de Melo, investigated 523 persons. He too verified the frequent presence of parasites in general and of ankylostomas in particular, in many cases lodged in the intestines of carriers who were themselves healthy with no apparent signs of ailment. In 1926, in a work published in the *Bulletin of the Society of Exotic Pathology*, in Paris, Froilano de Melo and his co-workers (4) established the following percentage of parasitic infestation after 596 stool analyses:

TABLE 1

Parasites	Percent
Amoeba	35
Flagellates	19
Blastocystis	16
Ascaris	75
Tricocefalus	48
Ankylostoma	32
Strongyloidis	7
Oxyuris	8
Taenia	2

In 1944, Pacheco de Figueiredo and Carlos Barbosa (5) examined 50 primary school children, between 6 and 9 years of age. The stool analyses yielded the following results:

TABLE 2

Parasites	Percent
<i>Worms:</i>	
Ascaris	74
Tricocefalus	64
Ankylostoma	24
Strongyloidis	2
<i>Amoeba:</i>	
Endamoeba coli (cysts)	14
Endamoeba coli (vegetative stage)	16
Endamoeba histolytica (cysts)	6
Endamoeba histolytica (vegetative stage)	4
Endamoeba Butschlii	2
<i>Flagellates:</i>	
Chilomastix	6
Blastocystis	6
Giardia	2
Trichomonas	6
Enteromonas	4
Charcot-Leyden crystals	6
Stool examination negative	3

In 1953, Almerindo Lessa and his co-workers (6) drew up statistics with reference to two sets of persons:

The first series comprised 282 hospitals patients and the second series of 50 outside persons without clinical symptoms. In the latter series the method followed for the investigation of *Endamoeba histolytica* was that of concentration with zinc sulphate (Faust). In the second series they found 46 percent of the individuals infested with *E. disenteriae*, while the result of the first series was as follows:

TABLE 3

Parasites	Percent relative	Percent total
Amoeba.	7.6	7.1
Ascaris	57.5	36.5
Tricocefalus	48.4	32.6
Ankylostoma	23.1	18.7
Trichomonas	2.2	2.1
Strongyloidis	2.9	2.8

NEW LABORATORIAL DATA

Going through the records of the Medical College Hospital of Goa, we found from the stool analyses of 2,136 persons suffering from various diseases, most of which were not intestinal, that 1,248 of them (58.4 percent) suffered from parasitic infestation, while 888 (41.5 percent) did not.

TABLE 4

Parasites	Percent relative	Percent total
Amoeba histolytica	27.7	16.2
Amoeba coli	2.6	1.5
Ascaris	58.5	34.2
Ankylostoma	25.0	14.6
Strongyloidis	2.0	1.2
Tricocefalus	26.6	15.6
Oxiuris	0.3	0.2
Flagellates	6.8	4.0

The percentage of Charcot-Leyden crystals was 6.5 and 3.8, respectively. 85 analyses were positive for flagellates, being 60 for Trichomonas, 15 for Chilomastix and Blastocystis and 10 for Lamblia. In one analysis of an African were found ova of schistosoma.

It was also found, from stool analysis of 1,248 persons, that in almost 40 percent of the cases the parasitic infestation was double, triple or more.

TABLE 5

Number of parasites	Percent
1.	59
2.	28.5
3.	10.1
4 or more.	2.2

As regards ankylostomiasis, in 70 patients observed in the Goa Medical College Hospital, the results of the stool analysis were as follows:

TABLE 6

Parasites	Percent
Ankylostoma only	24.4
Ankylostoma + amoeba	14.2
Ankylostoma + ascaris	41.8
Ankylostoma + tricocefalus	19.3
Ankylostoma + trichomonas	2.0

What is the significance of the presence of Charcot-Leyden crystals in faeces? For Knowles, McJunkine and Goltosch they are products of digestion or lysis of tissue matter, specially lipoid, by the specific and cytolytic action of amoeba. For Thomson and Robertson they are to be usually found in amoebic dysentery stools and they are of *great diagnostic value*. For Buecklers they are *very frequent* in cases of existence of *ankylostomas* or *strongiloidis* and, in decreasing order, in *ascaris*, *oxiuris* or *tricocefalus*. Manson-Bahr, on the other hand, insists on the danger of regarding them as a decisive element in the diagnosis of intestinal amoebiasis, as they are also to be found in malignant diseases of the rectum, in ulcerative and mucous colitis, in coccidiosis and in various helminthic infections.

Opinions thus differ.

We give bellow the parasitic index of 82 persons who had Charcot-Leyden crystals in their stool:

TABLE 7

	Number of persons	Percent
Parasites	41	50
Amoeba	19	23
Ascaris	27	33
Ankylostoma	6	7

In relation to the total number of analyses that were positive for parasites, the percentage is as follows:

TABLE 8

Amoeba	346	Charcot-Leyden	19	5.5 %
Ascaris	731	Charcot-Leyden	27	2.7 »
Ankylostoma	313	Charcot-Leyden	6	2 »

CLINICAL DATA

We make just a few observations on roundworm infection amoebiasis and ankylostomiasis.

a) Roundworm infection

Among ignorant people it is the greatest scourge, which, together with *dietary error*, contributes to increase mortality in early infancy. Acute and chronic enteritis, toxicosis, atrepsia — these are some of the more frequent manifestations, to which may be added the meningeal syndromes, bronchitis, asthma (with or without Löefflers syndrome), allergic dermatosis and, though rare, intestinal obstruction.

b) *Amoebiasis*

Its incidence goes beyond the results supplied by faecal examinations. If the acute forms may be easily diagnosed, the same is not the case with chronic forms (colitis, appendicitis, sprue, etc.), where anamnesis and stool examinations do not always reveal the cause. There are cases in which stool examination is positive for amoeba only at the third or fourth attempt! That is why, in doubtful cases, we lay down that anti-amoebic treatment should be given. Emetine takes pride of place in the treatment of hepatic localizations. We have not come across of urinary amoebiasis besides those described by Froilano de Melo (6). Pulmonary abscess is rare. The most frequent extra-intestinal localization is hepatitis. We have recorded 27 cases of liver abscess in a total of 2,136 patients (1.26 percent).

TABLE 9 — *Amoebic abscess of the liver*

	Percent
Men (21)	78
Women (6)	22
Antecedents with dysentery	13
<i>Amoeba histolytica</i> in stool	22
Cured	78
From 2 to 12 years	11
From 20 to 49 years	52
From above 50 years	37
Average leukocytosis (7,400 to 26,000 percent)	14,600
Average neutrophilia (52 to 92 percent)	80

c) *Ankylostomiasis*

Although there may be an apparently healthy carrier, this parasite has a most harmful action on under-nourished persons, giving rise to very serious clinical pictures: congestive heart failure, hypoproteinemic oedemas, polyneuritis, serous cachexia with ascites, etc.

We have observed cases of ankylostomiasis and amoebiasis associated with duodenitis and duodenal ulcer (7). But what is particularly alarming is the anaemia, which at times is the main symptom. How serious it can be may be seen from the following 70 observations (8):

TABLE 10

	Percent
With R.B.C. below 1,300,000 c m	6.32
With R.B.C. ranging from 1.3 to 2.6 millions	40.48
With R.B.C. ranging above 2.6 to 3.5 millions	20.24
With R.B.C. ranging above 3.5 to 4.5 millions	26.56
With R.B.C. ranging above 4.5 to 5 millions	6.32
<i>Hemoglobin percentage:</i>	
15 to 25 percent	10.12
26 to 35 percent	8.85
36 to 45 percent	29.93
46 to 55 percent	7.59
56 to 75 percent	26.56
76 to 85 percent	17.71
<i>Colour index:</i>	
0.5 to 0.6	8.85
0.6 to 0.8	16.44
0.8 to 1.0	32.89
1.0 to 1.5	12.65
1.6	29.93

CONCLUSION

From what has been said it follows that, in tropical countries, intestinal parasitosis has to be considered along with unbalanced diet as a factor contributing to the weakening of the population giving

rise to various ailments. A wide and thorough inquiry is called for with a view to determining the degree of incidence of parasitosis and to localizing the sources of ankylostomiasis.

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BROAD SPECTRUM ANTHELMINTHIC THERAPY WITH DITHIAZANINE¹

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In the classical treatise entitled *This Wormy World*, Stoll presented a careful estimate of the amount of parasitism of man by helminths. Over 2,250 million helminthic infections were harbored by a world population of 2,166 millions (1). In the light of Stoll's estimates of the enormous number of helminthiases, any improvement in the chemotherapy of these infections and in our knowledge of the mechanisms involved in the action of anthelmintics assumes increased significance.

The development of therapeutics with a wide range of antibacterial activity has stimulated a search for anthelmintics of comparable action against human intestinal helminthiases. Results of recent studies with dithiazanine demonstrate the effective broad spectrum of anthelmintic activity of this therapeutic against infections with important intestinal nematodes, e.g., *Trichuris trichiura*, *Strongyloides stercoralis*, *Enterobius vermicularis* and *Ascaris lumbricoides*. Presently available evidence indicates that dithiazanine also has some

¹ The investigations of the authors and their associates quoted in this review were conducted with the support of research grants from the National Institutes of Health and the Eli Lilly and Co.

anthelmintic activity against *Necator americanus*, *Taenia* sp., *Hymenolepis nana* and *Trichinella spiralis* adults.

NEW ANTHELMINTHIC

Dithiazanine is a polymethine dye which is designated chemically as 3,3'-diethylthiadicarbocyanine iodide. It is only slightly soluble in water and is not ordinarily absorbed from the intestinal tract to any clinically detectable degree. No dithiazanine iodide was detected in the urine of 25 patients who received 600 mg daily for 42 days (2). No evidence of hematopoietic, hepatic or renal damage has been detected in patients treated with therapeutic doses of dithiazanine iodide for periods up to 6 weeks (2). A specially coated tablet designed for slow release in the small intestine has been employed in most studies. The dye is excreted in the stool, to which it imparts a blue color.

ANTHELMINTHIC ACTIVITY IN ANIMALS

Studies indicate that dithiazanine possesses activity against hookworms, whipworms and ascarids of dogs, hookworms and ascarids of cats, pinworms of mice (3) and pinworm, *Strongyloides ratti* and *Hymenolepis nana* infections in rats (4) and *Trichinella spiralis* adults in rats (5). Clinical evaluation of dithiazanine has been undertaken by many investigators and the number of recorded cases treated totals 676 (6).

TRICHURIASIS

Substantial evidence of significant therapeutic effectiveness of dithiazanine for trichuriasis has been obtained (7, 8, 9). In one study of 201 patients with light or moderately heavy *T. trichiura* infections, cure rates in 5 groups treated for 1, 2, 3, 5 and 10 days with 200 mg *t.i.d.* were 3, 24, 77, 97 and 100 percent, respectively. Experience with treatment for whipworm infections characterized by diarrhea or dysentery has shown that both clinical and parasitic cure may be achieved with dithiazanine. Treatment for periods up to 10 or more

days may be required to obtain complete elimination of whipworms in patients with heavy infections (8a). The anthelmintic can be life-saving in severe trichuriasis (9).

ASCARIASIS

Ascaris infections, coincidentally, were eliminated in 66 percent of 47 patients, most of whom were being treated with exploratory dosages of dithiazanine for trichuriasis (8b). An egg count reduction of 96 percent occurred in 37 patients treated under similar conditions (8a). Massive infection with *A. lumbricoides* may be eliminated with dithiazanine without occurrence of intestinal obstruction or evidence of stimulation of the worms (9).

STRONGYLOIDIASIS

Twenty-five of 27 thoroughly studied cases of strongyloidiasis treated with dithiazanine have been *cured* with regimens of 5 to 21 days. Initially, a dosage of 200 mg *t.i.d.* for adults was employed (4). Subsequent studies now indicate that 100 mg *t.i.d.* for 21 days may be equally effective (10). The criteria for cure have been the absence of demonstrable parasites in extensive series of both stools and duodenal drainage fluids for periods up to a year or longer. Thorough examination of the duodenum of two patients treated for strongyloidiasis, who later expired from malignancy or other illness, revealed no evidence of persistence of infection (10). The necropsy study of these two patients and the elimination of *S. ratti* infection in 100 percent of animals treated with this anthelmintic, based upon both stool and autopsy examination, further supplement the evidence that *finally* an effective therapeutic for strongyloidiasis has been demonstrated adequately (4, 9, 10).

ENTEROBIASIS

A cure rate of 100 percent, based upon 7 consecutive negative post-treatment cellulose tape swabs, has been obtained in pinworm

infections with 100 mg of dithiazanine *t.i.d.* for 5 days (11). Evidence of activity of this compound against human pinworms is supplemented by its anthelmintic effect on murine pinworms (3, 4). Evaluation of the therapeutic efficacy of lower dosage and/or shorter treatment schedules for enterobiasis with dithiazanine appears to be indicated.

OTHER INTESTINAL HELMINTHS

A 65 percent reduction of the total egg counts in cases of heavy hookworm infection, with the recovery of *Necator americanus* adults, was obtained with 10 days of treatment with dithiazanine. A combination of 600 mg of dithiazanine and 1 or 2 g of tetrachloroethylene on 3 alternate days gave a 36 percent cure rate of heavy hookworm infections with a 97 percent reduction of the egg count in those cases in which elimination was not complete (9). The combination of these two anthelmintics appeared to have an additive or, possibly, a synergistic effect on *N. americanus*. Susequent studies with the same three dose regimen of these two anthelmintics have given the following cure rates: trichuriasis, 100 percent; ascariasis, 100 percent; and hookworm, 50 percent (12).

The data of two groups of investigators suggest that dithiazanine may be active against some cestodes, e.g., *Taenia* sp. (13) and *Hymenolepis nana* (2, 13). The number of cases treated is not sufficient to determine a valid therapeutic rate for these tapeworm infections.

ACTION ON HELMINTHS

Both immature and mature ascarids are expelled during therapy with dithiazanine. The worms are stained greenish-blue and are flaccid and non-motile upon passage. Rinsing and incubation result in a return of motility. In contrast, the effect of the drug on the motility of whipworms is not reversible. Incubation of *T. vulpis* from dogs for short periods in low concentrations of dithiazanine iodide resulted in an irreversible inhibition of anaerobic carbohydrate metabolism (14). The presence of the dye in the stools did not prevent embryonation of eggs of *T. trichiura*, *A. lumbricoides*, *N. americanus*

or *S. ratti*. It did not inhibit the motility of rhabditiform larvae of *S. stercoralis* and *S. ratti* or prevent their development to the filariform stage.

REACTIONS AND CONTRAINDICATIONS

Side effects during therapy of 429 cases by the authors and their associates were usually mild and transient and were manifested by anorexia, nausea, vomiting, abdominal cramps or diarrhea. The reactions were infrequent in adults and observed most commonly in small children who received high dosage of the drug. Patients with anemia, malnutrition, heart disease, nephrosis, pregnancy, emphysema, tuberculosis, malignancy and many other conditions have received the drug orally in therapeutic doses for helminthiases without ill effect.

SUMMARY

The principle of *effective* broad-spectrum anthelmintic activity against human intestinal helminth infections has been demonstrated by the therapeutic results obtained with dithiazanine. This new anthelmintic has significant activity against trichuriasis, strongyloidiasis, ascariasis, enterobiasis, *Necator americanus* and apparently other nematode and some tapeworm infections of man. It meets urgent needs for effective therapeutic for *Trichuris trichiura* and *Strongyloides stercoralis* infections. Extensive laboratory studies revealed no evidence of hematopoietic, hepatic or renal damage or of presence of the drug in the urine of patients who received the anthelmintic for 6 weeks. No serious reactions occurred in over 400 persons treated by the authors with a maximum of 600 mg daily for 1 to 42 days. No ill effects were observed in patients with anemia, malnutrition, pregnancy and numerous other conditions who received therapeutic doses of this drug for helminthiases. The polyvermicidal action of dithiazanine permits effective treatment of patients with single or multiple intestinal infections with several helminths of global significance.

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SPECIATION IN ECHINOCOCCUS (HYDATID)

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ABSTRACT

The almost universal distribution, the pleomorphic nature of the hydatid cyst, and the wide range of hosts both of the cyst and the tapeworm, have led to considerable confusion in speciation. This confusion has been still further complicated by the presence of the parasite in a wide variety of wild animals. While man is usually infected from the dog and the dog, in turn, from sheep, pigs, or cattle, other carnivores and other herbivores often act as domestic or sylvatic reservoirs. Investigations during the past five years on the wolf-deer sylvatic cycle in Canada have suggested that the Canadian species is serologically and ecologically distinct from that occurring elsewhere, and have drawn attention to the need for a much wider investigation on the speciation, immunology, and comparative pathology of echinococcosis in different parts of the world. This paper is a general discussion of our present state of knowledge on these subjects.

GENERAL DISCUSSION

PROF. DR. H. F. NAGATY (U.A.R.) — Piperazine salts have been tried by us in Cairo and were found effective against *Trichostrongylus*, *Heterophyes* and *Trichocephalus* besides *Ascaris* and *Enterobius*. Massive single dosage up to 6 g has been given against *Ascaris* with high cure rate. The work is published in *Lancet* and *Zeit. f. Tropenmedizin & Parasitologie*.

DR. EL BITASH (Egypt) — The wide spectrum drugs we were discussing are given on a long period of time from 5-10 days to be effective. In our country, where we treat at least 1-2 million people yearly, it will be rather expensive and not practical using such drugs. We hope we can find some drug as CCl_4 , which cures *Angylostoma* in one or two doses. This will be more practical in out-patient clinics.

DR. D. A. BERBERIAN (U.S.A.) — While at the American University of Beirut, Beirut, Lebanon, Doctors Turner, Dennis and I carried out immunization studies in sheep using hydatid fluid and hydatid sand from fertile cysts as antigen. We found that immunization does not prevent the establishment of cysts, but the cysts produced are degenerative cysts. In such cysts there is considerable calcification and the germinating membranes are sterile. In Lebanon hydatid cysts are most common in the lungs in cattle, and in the liver in sheep. Hydatid fluid from degenerate cysts is antigenically poor and gives negative or weakly positive Casoni reactions. We believe that degenerative cysts occur in those animals which have been sensitized previously by an early low grade infection.

DR. W. H. WRIGHT (U.S.A.) — I should like to ask Dr. Neghme what anthelmintic he employs against *Echinococcus granulosus* in dogs, the dosage and the results obtained.

DR. NEGhme (Chile) — We have used arecoline hydrobromide in doses of 4 mg per kilo of weigh, given by mouth. It is 90 percent (at least) successful on natural infections.

DR. A. NEGhme (Chile) — Los planteamientos hechos por el profesor Camerón con referencia a las variaciones larvales de *E. granulosus* solo pueden aceptarse como hipótesis de trabajo, pues le falta todavía la base experimental.

En Chile, en los últimos 20 años se ha venido observando un incremento de las localizaciones pulmonares de la hidatidosis humana, en desmedro de la hepática. Igual observación ha sido hecha en Uruguay (V. Perez Fontana) y en Argentina (A. Ferro). Talvez esto se deba al mejor diagnóstico de dichas formas por el uso cada día más extendido y frecuente de los rayos X.

Con referencia a los resultados obtenidos por el Dr. Swartzwelder con dithiazanina, mis colaboradores (Donckaster, Donoso F., Fai-guenbaum) los han confirmado en lo que se refiere a algunos gusanos nematodos. Pero la droga hasta ahora ha demostrado poca o nula eficacia contra los cestodes parásitos del hombre (*Taenia saginata*, *D. latum* y *Hymenolepis nana*).

DR. J. CLYDE SWARTZWELDER (U.S.A.) — As pointed out by one of the discussors, it would be ideal to have a broad spectrum intestinal anthelmintic which would be effective as a single dose treatment for mass therapy. However progress usually is achieved in small increments and the introduction of dithiazanine, the first effective broad spectrum intestinal anthelmintic represents a step toward that ideal. The length of time required for effective therapy with dithiazanine already has been reduced for strongyloidiasis. I believe that shorter treatment schedules for pinworm infection also may be equally as effective as the 5 day regimen which we employed. Data on mass therapy of whipworm infection, which I did not have time to present in this paper, indicate that the percentages of egg count reduction were 52, 88 and 99 percent respectively with 1, 2 and 3 days of treatment. Thus some steps have been taken already to obtain shorter courses of therapy.

The meeting was opened by the acting Chairman, Dr. Amador Neghme, in the absence of the appointed Chairman, Dr. Koyo Okabe. The Rapporteur was Dr. W. H. Wright and the Secretary was Dr. F. A. Carvão Gomes.

The following papers were heard and discussed.

THE USE OF INTRADERMAL AND INDIRECT HEMAGGLUTINATION TESTS FOR THE DIAGNOSIS OF VISCERAL LARVA MIGRANS¹

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and

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In 1952 Beaver *et al.* (1) demonstrated that a syndrome of chronic extreme eosinophilia with hepatomegaly in children was caused by tissue infection with larvae of the ascarid, *Toxocara*, probably *T. canis*, and suggested for it the term *visceral larva migrans*. They pointed out that the same or similar syndrome had been described many times before under different names and ascribed to other etiologies (4, 17, 21, 24). More recently, it has been recognized that other helminths than *Toxocara* may be responsible for the syndrome of visceral larva migrans (2).

At Charity Hospital of Louisiana, at New Orleans, high eosinophilia accompanied by hepatomegaly is frequently seen, particularly in children, and rarely is an etiologic diagnosis other than visceral larva migrans provable. To prove the diagnosis of visceral larva migrans it is necessary to demonstrate the presence of the worms

¹ Supported by Grant E-1159 of the National Institute of Health, U.S. Public Health Service.

in the tissues, preferably by liver biopsy. Since this procedure carries an inherent risk, investigations have been undertaken for the development of an immunologic test for the diagnosis of non-patent helminth infections such as larval toxocariasis.

INTRADERMAL TESTING WITH *TOXOCARA* AND *ASCARIS* ANTIGEN

The first studies undertaken involved intradermal testing for sensitivity to antigens prepared from *T. canis* and *Ascaris lumbricoides*. Aqueous extracts of adult worms and larvae (embryonated eggs) of both species were made. No difference in reaction between

TABLE 1 — *Results of intradermal tests with larval Ascaris antigen in persons with or without ascariasis*

Type of patient	Reaction		Total
	Positive	Negative	
With ascariasis	26	9	35
No ascariasis	18	22	40
Total, . . .	44	31	75

$$X^2 = 5.45 \quad p = 0.02$$

antigen made from adults or larvae could be noted. Table 1 shows results of skin tests using larval *Ascaris* antigen in 75 persons, 35 of whom had intestinal ascariasis and 40 of whom had neither present infection nor history of ascariasis. Twenty-six of the former and 18 of the latter gave positive reactions.

It was apparent that the intradermal test was not of value in diagnosing intestinal ascariasis. Of particular interest was the high incidence of positive reactions in persons with no history of ascariasis. When the 40 individuals with no history of ascariasis were regrouped according to residence in Northern or Southern United States (Table 2), the positive reactions were found to predominate in the persons from the South (16 out of 26). Only 2 from northern states

gave positive reactions. This suggests that these positive reactions may not be «false» positive, but rather due to old subclinical infections with *Ascaris* or other helminth infections with common antigens, since such infections are more likely to be contracted in the South.

TABLE 2 — *Results of intradermal tests with larval Ascaris antigen in persons without a history of ascariasis*

Origin of patient	Reaction		Total
	Positive	Negative	
Northern states	2	12	14
Southern states	16	10	26
Total. . .	18	22	40

$$X^2 = 10.22 \quad p = 0.001$$

When the 75 patients receiving intradermal tests with larval *Ascaris* antigen were grouped according to the presence or absence of peripheral eosinophilia (more than 400 eosinophiles per cubic millimeter)

TABLE 3 — *Relationship of peripheral eosinophilia to results with intradermal tests using larval Ascaris antigen*

Type of patient	Reaction		Total
	Positive	Negative	
With eosinophilia	34	12	46
No eosinophilia	10	19	29
Total. . .	44	31	75

$$X^2 = 9.83 \quad p = 0.005$$

(Table 3), it was found that of 46 patients with eosinophilia 34 had a positive reaction, while only 10 of 29 without eosinophilia were positive. The tendency toward positive tests with eosinophilia was statistically significant but not very strong.

Results using antigen made from *T. canis* larvae were about the same as when *Ascaris* larval antigen was used, i.e., cross reaction was almost complete in the skin test. Of 51 patients tested with antigens of both species, only 6 gave reactions with the two antigens that did not correspond (Table 4).

The total impression gained from these results is that intradermal testing is not likely to provide important help to the physician in

TABLE 4 — *Results of intradermal tests with both Toxocara and Ascaris larval antigens*

Tests with <i>Toxocara</i> antigen		Total	Tests with <i>Ascaris</i> antigen		Total
Positive	Negative		Positive	Negative	
28	1	29	28	1	29
5	17	22	5	17	22
33	18	51	33	18	51

$$X^2 = 26.71$$

$$p = 0.001$$

diagnosing visceral larva migrans because of the frequency of false positive reactions.

THE HEMAGGLUTINATION TEST FOR DIAGNOSIS OF NON-PATENT ASCARID INFECTIONS

Because of the reported successful use of an indirect hemagglutination (11, 13-15) technic in the diagnosis of helminthic infections, this type of test was applied to the present problem. The Middlebrook and Dubos test (18), as modified by Boyden (5) and Kagan (13-15), was used. This calls for treatment of erythrocytes with tannic acid in order to make a coating of antigen adhere to them. The coated cells are agglutinated by specific antibody.

A potent antigen was prepared from *Toxocara* (obtained from young dogs) and *Ascaris* (from children) by blenderizing the adult

worms in phosphate buffered saline pH 7.2 and extracting for 12 to 14 hours at 5°C in the same medium. After this the mixture was centrifuged at 850 X g for 30 minutes. The supernate was labeled whole worm extract and lyophilized. Ampoules of lyophilized extract were stored at room temperature and broken open for use as needed.

An *Ascaris* antigen of even greater potency was prepared according to a method similar to that of Campbell (7) and of Oliver-González (19). This material consists largely of polysaccharide containing a small amount of nitrogen. It was found that a 1:5000 dilution of this antigen gave a good titre in the hemagglutination test with known positive rabbit sera.

A polysaccharide fraction from *Toxocara* was not made in sufficient quantity to be used in the present studies. Hence throughout the remainder of this paper *Toxocara* antigen refers to the whole worm extract and *Ascaris* antigen to the polysaccharide.

SPECIFICITY OF THE HEMAGGLUTINATION TEST IN LARVAL ASCARID INFECTIONS IN RABBITS

Rabbits were immunized by giving doses of infective *Ascaris* or *Toxocara* eggs at intervals of 1 week to 5 months by stomach catheter. Seven doses were given, the first 3 consisting of 600 to 800 eggs and the subsequent 4 increasing in number of eggs to about 150,000. Rabbits were killed by the larger inocula if given too early in the series, and high titres were not developed if large inocula were not given at all.

Four rabbits infected with *Toxocara* larvae developed high titres of antibodies (1:320 to 1:1280) against *Toxocara* antigen, but only low titres (1:20 to 1:80) against *Ascaris* antigen. Four rabbits infected with *Ascaris* larvae developed high titres (1:5120) against *Ascaris* antigen, but very low titres (1:20 to 1:80) against *Toxocara* antigen. These results indicate that the test is specific enough to distinguish *Ascaris* and *Toxocara* larval infection in rabbits when the titre of homologous antibody is high.

RESULTS OF THE HEMAGGLUTINATION TEST OF HUMAN SERA¹

The syndrome of visceral larva migrans consists of marked eosinophilia, hepatomegaly, fever, pneumonitis, and hyperglobulinemia and is expected to occur in children between the ages of 1 and 5 who habitually eat dirt. The basic lesions are eosinophilic granulomata. Of these features, two appear most important in diagnosis — marked eosinophilia and hepatomegaly. [Even hepatomegaly has been found not to occur in mild infections (23).] For purposes of analysis, hepatomegaly and eosinophilia exceeding 30 percent have been arbitrarily taken as clinical criteria of visceral larva migrans. Eosinophilia of a lesser degree is not characteristic of visceral larva migrans.

Human sera were tested coming from several types of persons:

1. Clinical cases of eosinophilic diseases including some suspected of being visceral larva migrans.
2. Cases of known infection with parasites other than *Toxocara*.
3. Individuals not known to have parasitic infections or eosinophilia.

Serum was obtained from patients mainly at the Charity Hospital of Louisiana, at New Orleans, Lallie Kemp Hospital, Independence, La., and the Tulane Tropical Medicine Clinic. Physicians inquiring regarding immunologic diagnosis of visceral larva migrans have been encouraged to send in serum for testing. As a result, specimens have been received from cases in various parts of the United States and several foreign countries.

Sera were tested from 78 persons who had complete physical examinations, blood counts and stool examination. Chest X-ray and additional clinical laboratory studies were done on the majority. Of these patients, 19 had eosinophilia exceeding 30 percent (henceforth referred to merely as eosinophilia); 13 had eosinophilia without hepatomegaly; 4, hepatomegaly without eosinophilia; 42, neither.

¹ For additional data see JUNG, R. C., and PACHECO, G. — «Use of hemagglutination test in visceral larva migrans», *Am. J. Trop. Med. Hyg.*, 9: 185-191, 1960.

Hemagglutination tests of sera from patients with neither hepatomegaly nor eosinophilia usually gave titres below 1:320 with *Ascaris* antigen and below 1:160 with *Toxocara* antigen. Only 6 of the 42 sera gave titres with either *Ascaris* or *Toxocara* antigen or both higher than 1:320 and 1:160 respectively. Tentatively titres below these levels have been considered as not clinically significant (see Table 5).

Of 4 patients with hepatomegaly but not eosinophilia, 3 had titres not significant and 1 had a high *Ascaris* titre. On the other

TABLE 5 — Results of hemagglutination tests in 78 patients using *Ascaris* and *Toxocara* antigens

Clinical features	<i>Ascaris</i> — <i>Toxocara</i> —	<i>Ascaris</i> + <i>Toxocara</i> —	<i>Ascaris</i> — <i>Toxocara</i> +	<i>Ascaris</i> + <i>Toxocara</i> +	Totals	Rate positive
Eosinophilia 30 0/0. . . Hepatomegaly	3	6	4	6	19	16/19
Eosinophilia 30 0/0. . . No hepatomegaly	7	3	2	1	13	6/13
Eosinophilia 30 0/0. . . Hepatomegaly	3	1	0	0	4	1/4
Neither.	36	3	2	1	42	6/42
Total. . .	49	13	8	8	78	29/78

Ascaris + = titre > 1/320

Toxocara + = titre > 1/160

hand, 7 of 13 patients with eosinophilia, but not hepatomegaly, had titres with both *Ascaris* and *Toxocara* that were not significant.

Sixteen of 19 patients (84 percent) with eosinophilia and hepatomegaly had high titres against either *Toxocara* or *Ascaris* antigen or both. Of the 3 patients with non-significant titres, 1 ran a course not characteristic of visceral larva migrans and eventually died. Examination of tissue, including digestion of the entire lungs and liver, revealed the presence of eosinophilic granulomatosis but no helminth larvae.

It thus appears that the hemagglutination test with *Ascaris* or *Toxocara* antigen tends to be positive in high titres when there are clinical features of visceral larva migrans. The approximately 20 per cent low titres or negatives in these patients may be due to failure of the test or because the syndrome is sometimes caused by other agents.

Six of 42 individuals without features of visceral larva migrans had high titres which may have been due to failure of the test (non-specific agglutination), subclinical infection or antibodies resulting from intestinal ascariasis or its preceding larval migration. Such a rate of subclinical infection is reasonable since these patients were all drawn from groups in which the exposure to infection with *Ascaris* and *Toxocara* is higher than the average (children of low income families). Four of the 6 had intestinal ascariasis.

TABLE 6—Results of hemagglutination tests in patients with various intestinal helminth infections using *Ascaris* and *Toxocara* antigens

Infection	<i>Ascaris</i> = <i>Toxocara</i> —	<i>Ascaris</i> + <i>Toxocara</i> —	<i>Ascaris</i> — <i>Toxocara</i> +	<i>Ascaris</i> + <i>Toxocara</i> —	Total	ate +
Ascariasis.	20	2	1	1	24	4/24
Trichocephaliasis. .	10	0	1	0	11	1/11
Strongyloides . . .	5	0	0	0	5	0/5
Hookworm	4	0	0	0	4	0/4
Total. . .	39	2	2	1	44	5/44

Ascaris + = titre > 1:320

Toxocara + = titre > 1:160

Of the 42 individuals with neither eosinophilia nor hepatomegaly 24 had intestinal ascariasis, 11 trichocephaliasis, 5 strongyloidiasis and 4 hookworm infections. Results with hemagglutination tests in these groups is shown in Table 6. There was evidence of cross reaction only in 2 patients with ascariasis and 1 with whipworm.

DISCUSSION

Although it has long been known that persons with ascariasis may have a positive intradermal reaction with *Ascaris* antigen and it has even suggested that the skin test be used for routine diagnosis of ascariasis (6), the usefulness of this method has been impaired not only by cross-reactivity between the antigens of *A. lumbricoides* and other helminths, but also by the long persistence of skin sensitivity after an infection has ended (9). On the other hand, serologic tests, although more complicated, promise to be more specific because they are subject to quantitation. Antibodies to ascarid infection have been detected by precipitation (8, 12), complement fixation (16), hemagglutination (15) and bentonite flocculation (22) technics. Of these the hemagglutination test has been found to be sensitive and specific in other helminth infections and adaptable to the routine diagnostic laboratory.

While the indirect hemagglutination test is demonstrably diagnostic in experimental ascarid infection in rabbits, the evaluation of the technic in human infection is much more difficult. Partly this is because human beings may not produce titres of antibodies as high as do rabbits, but the principal obstacle to the evaluation of any diagnostic test in visceral larva migrans is the paucity of proved cases. Until a satisfactory number of such have been accumulated it will be necessary to compare the results of the hemagglutination test with the clinical features of the disease.

In New Orleans the syndrome characteristic of visceral larva migrans — fever, hepatomegaly, marked peripheral eosinophilia, pneumonitis and hyper-globulinemia in children 1 to 4 years of age — is not readily attributable to other causes. That infection with *Capillaria hepatica* may be the origin of such an illness in the southern United States has been proved (20), and the possibility remains that other non-patent helminthic infections may produce similar pictures (2). Such other causes of hepatomegaly with eosinophilia may explain the failure of the hemagglutination test in 3 of 19 cases with these features in the present study.

Elsewhere in the world other diseases may commonly produce some of the signs and symptoms of visceral larva migrans, e.g.,

schistosomiasis. Other eosinophilic diseases with overlapping features, e.g., tropical pulmonary eosinophilia, may be of considerable importance. Non-patent infection with *Ascaris* (3) and with *Filaria* (10) have been incriminated in tropical pulmonary eosinophilia. Certainly we may expect visceral larva migrans to occur in these sites as well as in the United States, but recognition on a purely clinical basis must be very difficult because of the greater possibility of confusion with similar diseases. In such situations a serologic test like the indirect hemagglutination technic will be of particular value, especially when suitable antigens are available for most of the other worms producing non-patent human infections.

SUMMARY

1. Intradermal testing with *Ascaris* and *Toxocara* antigen has been shown to be of little value in diagnosis of visceral larva migrans.
2. Antigens have been prepared and a hemagglutination test standardized which are capable of giving reproducible results in the detection of antibodies resulting from larval *Ascaris* and *Toxocara* infection.
3. The hemagglutination test is specific and sensitive enough to detect and distinguish *Toxocara* and *Ascaris* larval infections in rabbits.
4. The presence of eosinophilia over 30 percent with hepatomegaly in patients tested was likely to be associated with high titres of antibody against *Ascaris* or *Toxocara* or both.
5. No significant cross reaction with *Trichocephalus*, *Necator* and *Strongyloides* infections was detected by the hemagglutination test.

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ECHINOCOCCUS MULTILOCCULARIS INFECTION

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The investigation of alveolar hydatid disease of man had its beginning more than a century ago, when Virchow (1855) reported that certain cases of apparent malignancy of the liver were caused by the invasive growth of a larval cestode of the genus *Echinococcus*. In recognition of the unusual morphological characteristics of this larval cestode, Leuckart (1863) applied to it the name *Echinococcus multilocularis*.

For about a century following Virchow's discovery, the specific identity of this cestode remained controversial. Many investigators, among whom Dévé and Dew were especially prominent, believed that alveolar hydatid disease resulted from the anomalous development of the larval *E. granulosus* (Batsch, 1786). As recently as 1953, Dew supported this concept in his discussion of variation in the development of the larval *E. granulosus*. Other workers, among whom Posselt was outstanding, were convinced that alveolar hydatid disease was caused by a distinct species of *Echinococcus*. Posselt defended this hypothesis in a large series of published papers, but his findings were generally not accepted. Among the more recent proponents of Posselt's dualistic concept were Henschen and Bircher, who stated (1945) that the cestode causing alveolar hydatid disease differed from *E. granulosus* in the dimensional proportions of its rostellar hooks, and in the form of the gravid uterus.

In 1949, the writer undertook the investigation of hydatid disease in Alaska, where *E. granulosus* was found to have a wide distribution. It was soon recognized, however, that another species of *Echinococcus*

existed on St. Lawrence Island, in the Bering Sea (Rausch, 1952; 1953). This cestode differed morphologically and ecologically from *E. granulosus*, which does not occur on St. Lawrence Island. Moreover, it was determined that the larval cestode caused alveolar hydatid disease in man. This cestode was described by Rausch and Schiller (1954) as *E. sibiricensis*, and it was believed to be conspecific with that causing alveolar hydatid disease in Eurasia (Rausch, 1952; Rausch and Schiller, 1956).

The findings in Alaska led Prof. Hans Vogel, of the Nocht Institut für Schiffs- und Tropenkrankheiten, to undertake anew the investigation of alveolar hydatid disease in Germany. In the Schwäbische Alb, where the disease is endemic, Vogel (1955) discovered a cestode morphologically and ecologically identical with *E. sibiricensis*. He pointed out at this time that Leuckart's name had priority, should it be confirmed that the cestodes were conspecific. Through detailed experimental studies, and the comparison of adult cestodes collected from canine animals in Alaska and Germany with those obtained by feeding material of human origin to dogs, Vogel (1957) established beyond doubt that *E. multilocularis* is the cause of alveolar hydatid disease in man. It was also determined that the Alaskan and European forms of *E. multilocularis* differ in minor details of structure, host-specificity, and pathogenicity; consequently, Vogel (1957) proposed that the Alaskan form be regarded as a subspecies of the European species.

Some workers appear reluctant to accept the conclusions outlined above. Poole (1957: 427) stated: «The type of hydatid found in wild rodents was called alveolar hydatid, but it bears no resemblance to the human alveolar type.» He did, however, express the opinion that «There is a probability that this parasite is different from that usually associated with dogs, herbivores and man as found in Canada.» Leikina (1957) reviewed the status of the problem in the Soviet Union and considered premature the conclusion that *E. sibiricensis* and *E. multilocularis* are conspecific, although she agreed that *E. sibiricensis* is the cause of alveolar hydatid disease in man. Leikina's attitude stemmed from failure to understand the relationships of multicystic larvae from ruminants, a point which has also confused other workers (cf. Dew, 1953).

It is the purpose of this paper to summarize briefly the information available on the bionomics of *E. multilocularis* and on the disease it produces in man. The literature on this subject is voluminous, and space permits the citing of only a few of the most relevant references here.

HOST-RELATIONSHIPS OF *E. MULTILOCULARIS*

In nature, a canid and a microtine rodent are required for completion of the life cycle of *E. multilocularis*. Some of the more important hosts are species which have a circumpolar distribution.

IN NORTH AMERICA. — The arctic fox, *Alopex lagopus* Linnaeus, is the most important final host of *E. multilocularis* in Alaska. Naturally infected red foxes, *Vulpes vulpes* Linnaeus, have been collected (Rausch, 1956), but the difficulty with which they are infected experimentally indicates that they are relatively unimportant. Naturally infected dogs have been found on St. Lawrence Island. It has been determined experimentally that the cestodes develop normally in the wolf, *Canis lupus* Linnaeus, and in the grey fox, *Urocyon cinereoargenteus* (Schreber), but they have not been found to produce eggs in the coyote, *Canis latrans* Say.

The larval cestode has been recorded from two species of microtine rodents on St. Lawrence Island. These are the red-backed vole, *Clethrionomys rutilus* Pallas, and the tundra vole, *Microtus oeconomus* Pallas. Thomas *et al.* (1954) and the writer have also found infected shrews, *Sorex tundrensis* Merriam, on St. Lawrence Island. Infected rodents have not been observed on the mainland of Alaska, although the strobilar stage has been collected there (Rausch, 1956). Alveolar hydatid disease has been diagnosed in an Eskimo from Kotzebue, however. Several species of rodents have been infected experimentally in Alaska.

IN EURASIA. — On Bering Island, near the coast of Kamchatka, Afanas'ev (1941) found *E. multilocularis* in both arctic foxes and red-backed voles. Farther south, Ishino (1941) collected an infected vole, *M. oeconomus*, on Simushir Island, in the Middle Kuriles. On Rebun Island, Ambo *et al.* (1954) found an infected cat, and Yamashita *et al.* (1955) collected the cestode from dogs. Using material of Alaskan origin, Yamashita (personal communication) successfully

infected the following species of rodents: *Clethrionomys rutilus mikado* (Thomas), *C. rufocanus bedfordiae* (Thomas), *Microtus montebelli* (Milne-Edwards), *Meriones unguiculatus kurauchii* Mori, and *Apodemus g. geisha* (Thomas).

In Siberia, *E. multilocularis* has been reported from an Azerbaidzhan fox, and from an arctic fox on Kil'din Island (Leikina, 1957). Cestodes differing morphologically from *E. granulosus* were recorded also from foxes in the Omsk Oblast'. According to Leikina, larvae of *Echinococcus* have been found in red-backed voles from the Viliuisk region of the Yakutsk ASSR, and in a vole, *M. oeconomus*, from the Barabinsk region of the Novosibirsk Oblast'. Spasskii *et al.* (1951) recorded larvae from muskrats, *Ondatra zibethicus* (Linnaeus), from Kzyl-Orda and from the Kurgansk Oblast'. The latter were described specifically as alveolar. Vsevolodov (1953) made a detailed study of a muskrat having a generalized alveolar infection; this animal had been collected by an expedition of the Zoological Institute of the Academy of Science, Kazakh SSR. Spasskii *et al.* (1952) reported *E. granulosus* from 50.9 percent of 51 red foxes, *Vulpes v. daurica* Ognev, from the region of Lake Baikal. In view of the conclusions of Matoff and Jantscheff (1954) that *E. granulosus* does not develop normally in red foxes, these cestodes might have been *E. multilocularis*.

In Germany, Vogel (1955) found *E. multilocularis* in 4 of 11 red foxes from the region of the Schwäbische Alb, and the larvae in 4 of 260 voles, *Microtus arvalis* Pallas. Vogel (1957) also infected experimentally several species of microtine rodents indigenous to central Europe.

THE DISTRIBUTION OF *E. MULTILOCULARIS*

IN NORTH AMERICA. — Surveys thus far completed indicate that *E. multilocularis* is distributionally restricted in Alaska to the tundra zone. It may occur in arctic Canada, but this has not yet been determined. According to Miller (1953), cestodes of the genus *Echinococcus* have been collected from an arctic fox on Banks Island. Gibbs (1957) discussed the role of rodents in the epidemiology of hydatid disease in the Mackenzie River basin. He examined some wild rodents, but his work consisted mainly of unsuccessful attempts to infect such mammals experimentally with the eggs of *E. granulosus*.

James and Boyd (1937) reported a fatal case of alveolar hydatid disease in a 54-year-old resident of the Province of Manitoba. Although this man had immigrated from Iceland at the age of 7 years, it is possible that this was an autochthonous infection.

At the present time, the apparent absence of *E. multilocularis* from the United States and Canada cannot be adequately explained, since conditions for the survival and perpetuation of this cestode appear to be favorable.

IN EURASIA. — *E. multilocularis* was introduced on Bering Island with voles from the Siberian mainland (Stejneger, 1884; Barabash-Nikiforov, 1943; Johansen, 1950). It probably was introduced in the Middle Kuriles with foxes from Bering Island, and became established on Rebun Island following introduction of red foxes from the Middle Kuriles (Rausch and Yamashita, 1957).

E. multilocularis is widely distributed in the Soviet Union. Semenov (1955) reported a case from as far northeast as the region of Nizhne-Kolymsk. Leikina (1957: 150) stated that human infection «... is registered in West Siberia, in some regions of East Siberia, mainly in the Yakutsk ASSR, in Tartar and Bashkir ASSR and in separate regions of Kazakhstan, in other words, in the northeast section of the Soviet Union.» Pavlovskii (1946) stated that cases are observed annually in Irkutsk. There are also records in the literature of the occurrence of the disease much farther to the west (Henschen and Bircher, 1945).

In Europe, human cases have been diagnosed in northern Switzerland, in the French Jura, and throughout southern Germany, including especially Württemberg, Baden, and Bayern. According to the literature, *E. multilocularis* seems to be rare or absent in eastern Europe.

The approximate geographic distribution of *E. multilocularis* in North America and Eurasia is shown in Fig. 1. The few reports of alveolar hydatid disease in other parts of the world have not been considered here.

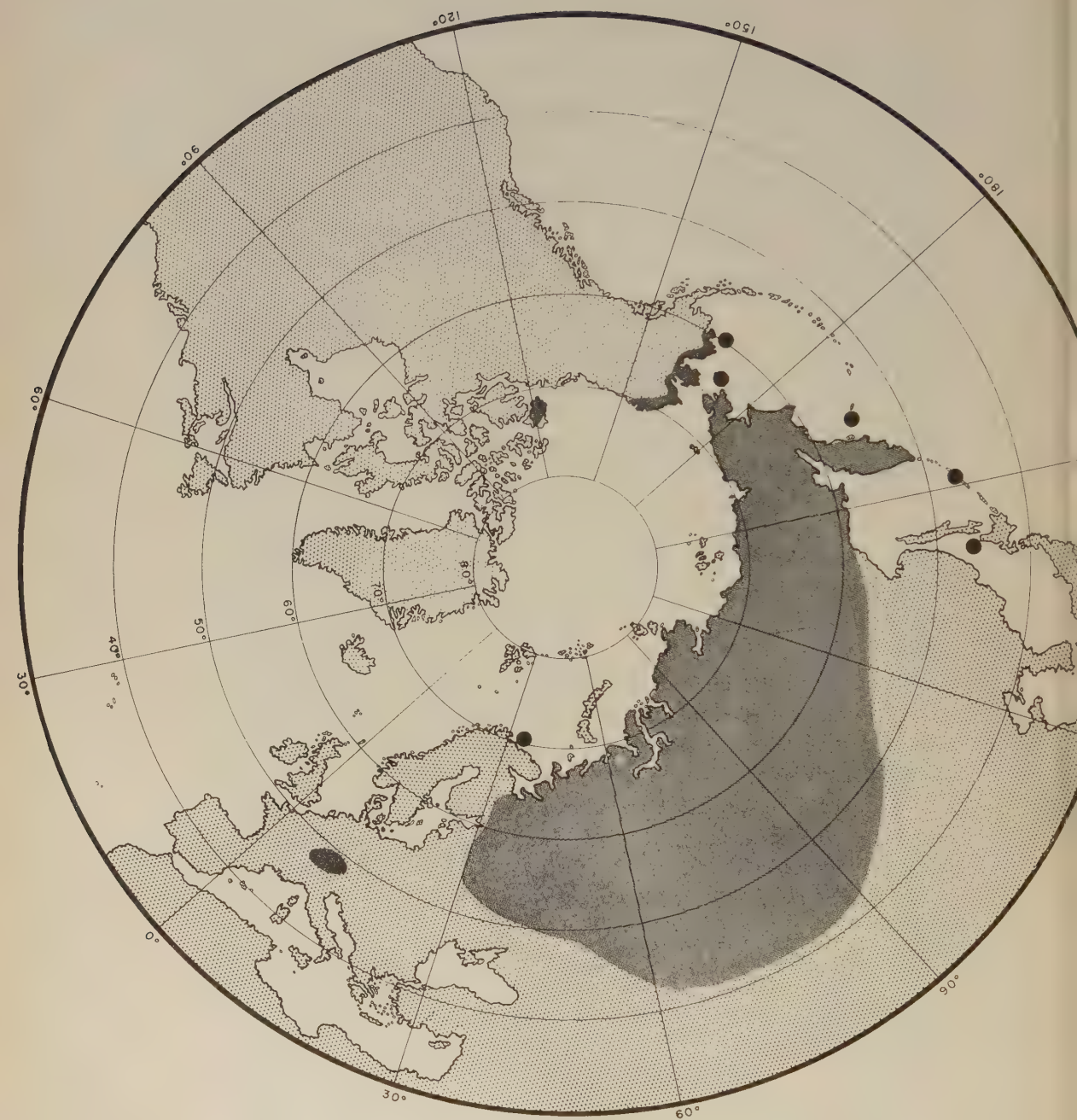


Fig. 1 — Approximate distribution (dark areas and circles) of *Echinococcus multilocularis* based on records in the literature

EPIDEMIOLOGY

In agricultural regions the natural cycle involves voles and foxes, but cats and dogs may replace foxes as the final host. Human infections may result when fruits and vegetables contaminated by the feces of canine animals are consumed. Close association with infected cats and dogs is especially dangerous. Yamashita (1956) expressed the opinion that contaminated water supplies might be an important source of infection on Rebun Island.

In northern regions, including Alaska and northeastern Siberia, infections may result from the careless handling of foxes which are trapped for their fur. However, it appears that the infection may be transmitted most frequently from sledge dogs. In Eskimo villages in Alaska, these dogs are very numerous and the environs of such villages are always grossly contaminated by their excreta. Eggs are undoubtedly brought into dwellings on soiled implements and footwear.

ALVEOLAR HYDATID DISEASE IN MAN

PATHOGENESIS. — After escaping the eggs in the small intestine, the onchospheres of *E. multilocularis* penetrate the intestinal wall and make their way to the liver by means of the portal circulation. In experimentally infected voles, the larvae penetrate the walls of the interlobular veins and enter the hepatic lobules, where growth begins (Rausch, 1954). The early stages of larval development in man are unknown. The larvae proliferate by exogenous budding, but the rate of growth in man appears to be much slower than in the natural hosts. According to Yamashita *et al.* (1955), the first human cases on Rebun Island were diagnosed about 12 years following the introduction of the parasite.

The liver is the organ most commonly affected in man. Dardel (1927) studied 102 cases in Switzerland, and found that more than 90 percent of these were hepatic infections. His comparative data on the localization of *E. multilocularis* and *E. granulosus* are summarized in the table here included. In the liver, the degree of pathogenicity is to a considerable extent related to the location of the infection.

When the larvae are more peripheral and superficial, the effect is less severe than when they are situated near the major bile ducts and blood vessels.

According to Posselt (1931) and Henschen and Bircher (1945), the right lobe of the liver is most commonly involved. In advanced

*A comparison of the localization and pathogenicity of the larvae of E. multilocularis and E. granulosus in man*¹

Locus of infection	<i>E. multilocularis</i> Number of cases : 102	<i>E. granulosus</i> Number of cases : 100
Liver	95	78
Lungs	1	9
Spleen	1	5
Kidneys	1	1
Brain	2	—
Bone	1	—
Abdominal wall	1	1
Ovary	—	1
Peritoneum and omentum	—	2
Parametrium	—	1
Thyroid and neck	—	2
Outcome	Number of cases : 102	Number of cases : 100
Died.	84	35
Treatment successful	2	43
Unknown	7	1
Found accidentally at autopsy	9	21

¹ After Dardel (1927).

hepatic infections, the pathological changes evoked are essentially those characteristic of portal hypertension. It is not unusual for the parasite to invade the gall bladder, the bile ducts, the larger blood vessels, and other organs. They sometimes rupture into the abdominal or thoracic cavities. When blood vessels are invaded, metastatic distribution of larval elements may result. Dardel (1927) observed 10 cases of metastasis when the primary infection was in the liver; 7 of these

were in the lungs, and 3 were in the brain. Primary foci other than in the liver are rare.

Man is a relatively unsatisfactory host for the larva of *E. multilocularis*, for, as the larval mass increases in size, the central portion dies and undergoes degeneration, and only the peripheral layer in close contact with the tissue of the host is able to survive. This peripheral layer continues its invasive proliferation, and usually encloses a large cavity containing purulent material and debris. The larval tissue consists mainly of subgerminal elements; few or no scolices are produced in most human infections.

The pathogenesis of alveolar hydatid disease has been discussed in detail by Posselt (1925; 1931), Henschen and Bircher (1945), and others.

SYMPTOMATOLOGY AND DIAGNOSIS. — *E. multilocularis* infection gives rise to a chronic, afebrile disease characterized by hepatomegaly, and with icterus and ascites in the terminal stages. The clinical signs are in general those typical of intrahepatic portal hypertension.

The earlier stages of hepatic infection appear to be more or less asymptomatic, although there are exceptional cases in which deterioration of physical condition is rapid. The appetite usually remains good, and the ability to work is little impaired. As the disease progresses, there is marked enlargement of the liver, enlargement of the spleen, ascites, and increasing icterus. In experimentally infected rodents, it is not unusual for the liver to increase in size until it comprises more than 50 percent of the total body weight before normal physiological functions are much affected. In man, the liver is usually quite hard in consistency. Clinically, it is difficult to differentiate alveolar hydatid disease from carcinoma of the liver. Several investigators have stated that an unusually high sedimentation rate (40 mm and more per hour) is characteristic of this disease. The clinical diagnosis of alveolar hydatid disease has been discussed recently by Henschen and Bircher (1945), Vogel and Minning (1952), and others.

Röntgenological diagnosis is sometimes possible if calcification has occurred (Friedrich, 1940). The calcified areas usually have a reticulate or granular appearance in röntgenograms.

Immunological tests have commonly been applied as an aid to the diagnosis of this disease. The Casoni intradermal test and the

complement fixation test have been most frequently used, but there is disagreement as to their reliability. Henschen and Bircher (1945) considered the intradermal test to be more accurate than the complement fixation test, but pointed out that false positive reactions are sometimes obtained. Using an antigen prepared from alveolar larvae, about twice as many positive intradermal reactions were obtained among Eskimos on St. Lawrence Island as were obtained from the complement fixation test using fluid from *E. granulosus* cysts for the antigen (Rausch and Schiller, 1956). More recently, Dr. G. F. Otto and his coworkers attempted to evaluate the significance of the relatively high rate of positive reactions obtained among Eskimo and military personnel on St. Lawrence Island. Using antigens prepared from alveolar larvae and from cyst-fluid, including fractions of the latter, it was found that a relatively high rate of positive reactions was obtained by both methods. When the two tests, using the same antigens, were applied to a control group of unexposed military personnel in the United States, a similar high rate of positive reactions was noted (Otto *et al.*; unpublished data). Hence, at the present time, it appears that these tests are not reliable for the diagnosis of alveolar hydatid disease.

Dr. I. G. Kagan and his coworkers (unpublished manuscript) have recently completed an evaluation of the hemagglutination and flocculation tests in the diagnosis of hydatid disease. They concluded that both methods are superior to the complement fixation test, but the hemagglutination test is the more accurate. Additional work is needed to determine the value of this latter test in the diagnosis of alveolar hydatid disease.

At times, even the *post mortem* diagnosis of alveolar hydatid disease is difficult, and pathologists unfamiliar with it are liable to make incorrect diagnoses. The latter is especially true in the United States, where the disease has been rarely observed. Vogel and Schumacher (1957) found that the periodic acid-Schiff reaction stains the larval membranes an intense red; this method makes possible the recognition of small vesicles and thin membranes which otherwise could be overlooked.

TREATMENT. — The curative treatment of alveolar hydatid disease consists of the surgical extirpation of the portion of the liver or other organ involved. Such treatment is often practicable during the earlier

stages of the infection, but advanced cases are usually inoperable. Much depends upon the location of the infection. When the major bile ducts and vessels are involved, or when multiple foci exist, resection is usually impossible. The best results are obtained when a single mass is so located as to permit a partial hepatectomy.

Most cases are far advanced when diagnosed, and the prognosis is then quite unfavorable. Of 25 cases from Rebus Island, 15 terminated fatally (Rausch and Yamashita, 1957). Semenov (1954) reviewed 152 cases treated by surgeons in the Soviet Union. Of these, 108 (71 percent) were inoperable; 21 terminated fatally after surgical treatment; at least 12 were considered to have been cured. The remainder either refused surgery, or the outcome was unknown. In addition to these, Semenov personally treated 65 cases; excepting one case in which the spleen was involved, all were hepatic infections. Forty-two cases were inoperable, and 2 patients died following exploratory laparotomy. Twenty patients had radical surgery; 15, including the one with the infected spleen, recovered, and 5 died. Thus, treatment was successful in about 24 percent of the cases. Dardel (1927) reported 102 cases; 84 terminated fatally, 2 were successfully treated, the outcome on 7 was unknown, and 9 were found accidentally at autopsy.

Surgical procedure in the treatment of alveolar hydatid disease of the liver has been discussed by Hosemann *et al.* (1928) and by Henschen and Bircher (1945). In other organs, surgical procedure is similar to that required for the local extirpation of a malignant tumor.

PREVENTIVE MEASURES

Alveolar hydatid disease in man can be prevented at the present time only by the establishment of hygienic practices effective in precluding the ingestion of the infective eggs of *E. multilocularis*. Since the infection of man takes place only through direct or indirect contact with animals in which the adult cestodes occur, the appropriate control of dogs and cats is especially important. An anthelmintic efficacious against *E. multilocularis* in such animals is not known at present.

In conclusion, it is emphasized that further research is required (1) to gain a better understanding of the epidemiology of alveolar hydatid disease to allow the development of adequate preventive measures, (2) to produce an anthelmintic effective against the adult cestode in dogs and cats, and (3) to develop techniques which would permit the diagnosis of this disease in man early enough for successful treatment.

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THE PUBLIC HEALTH SIGNIFICANCE OF PARAGONIMIASIS IN THE FAR EAST

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Throughout most of their existence, people in Asia live between the risks of dietary deficiencies, primarily in animal proteins, and the risks of helminthic infections deriving from the ingestion of improperly cooked freshwater fish and crustaceans. The two most common helminthic infections acquired in Asia through the ingestion of improperly cooked freshwater products are clonorchiasis and paragonimiasis. This summary is concerned with the latter, since information available on its public health significance is much more limited.

Stoll (1947) estimated that there were approximately 3,200,000 individuals infected with paragonimiasis in Asia. In the light of recent investigations conducted with the aid of improved diagnostic means, indications are that this figure represents only a minimum and that a much higher estimate of the true prevalence of this disease is more realistic. Recent epidemiologic investigations carried out with the aid of intradermal tests (Yokogawa *et al.*, 1955) have revealed that in Japan this disease is present in all but 7 prefectures. In 4 prefectures, between 10 and 20 percent of the population was found to be infected (Yokogawa, 1958) (Fig. 1). The prevalence of paragonimiasis in Korea is considerably greater. A preliminary survey carried out by the Police Office of the Korean Government (1924) revealed that paragonimiasis is endemic in every prefecture in Korea, with rates up to 23 percent of the population (Fig. 2). These figures were obtained by single sputum examinations of people in the areas surveyed. Considering that eggs are found in single sputum exami-

nation in less than 50 percent of the people infected with *P. westermani* (Yokogawa and Awano, 1956; Nishimoto and Sakamoto, 1957; Sadun *et al.*, 1958), its prevalence is probably considerably greater than indicated. In the course of a preliminary joint investigation, it was found that 3 out of 4 individuals tested in some villages on Cheju-Do Island off the coast of Korea were positive reactors for paragonimiasis.

In mainland China, paragonimiasis is endemic in Chekiang province, especially in Shaoshing district. Sporadic human cases and

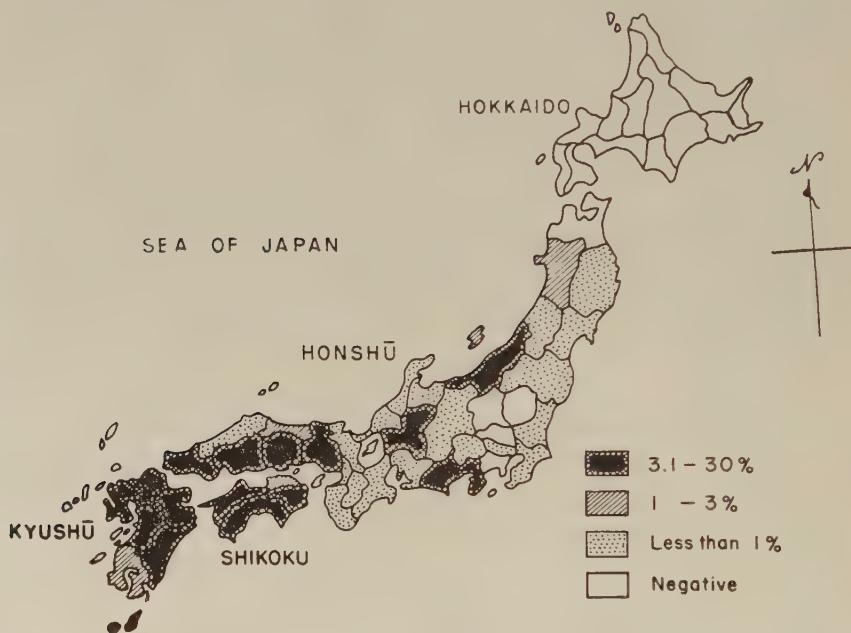


Fig. 1 — Distribution of human paragonimiasis westermani in Japan

infections in domestic animals have been reported also from other provinces. In Formosa, paragonimiasis is endemic among the aborigines in the mountains of Hsinchu and Miaoli and among the Chinese from those regions of the mainland where consumption of raw or improperly cooked fresh-water crabs is prevalent. The Japanese had prohibited the sale of crabs belonging to the genera *Potamon* and *Eliochior*. These measures, together with the widespread health education campaign carried out in endemic areas, brought about a progressive decrease in

the incidence of paragonimiasis. However, after World War II, these crabs are freely consumed, especially by the large number of Chinese who migrated from the mainland. This, and the relaxation of hygienic

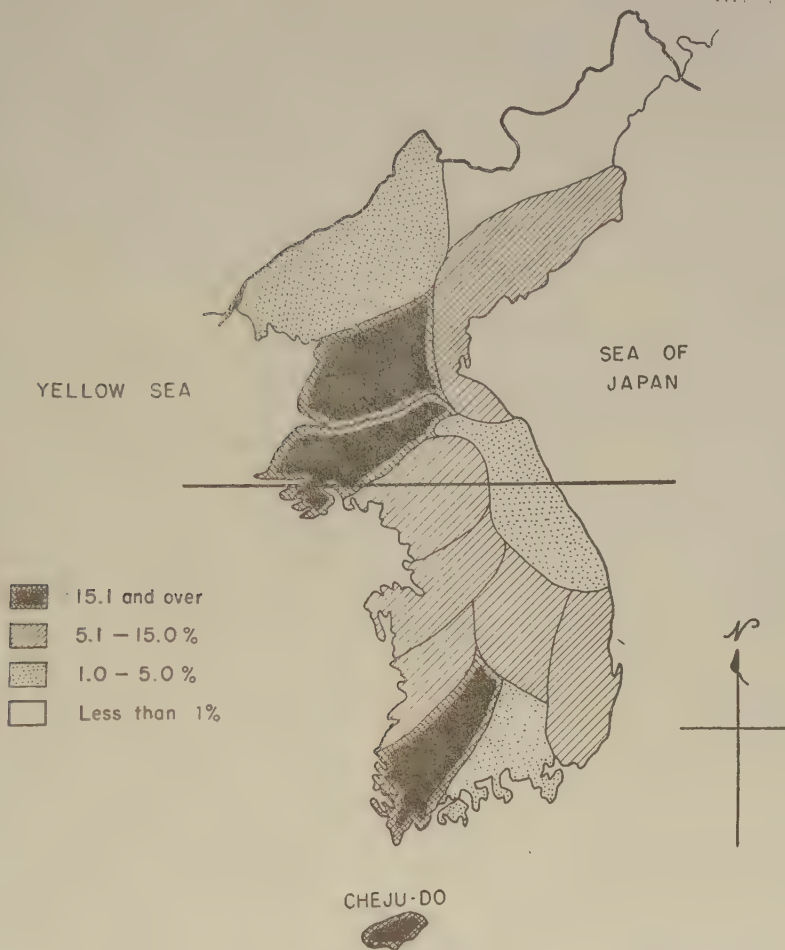


Fig. 2 — Distribution of human paragonimiasis westermani in Korea

habits in the general population have brought about a marked increase in the infection rate (Khaw, 1958; Huang, 1958).

Although only scattered information is available, it is evident that endemic foci are present also in South East Asia. Only last

year, an endemic area in Thailand was recognized for the first time (Vajrasthira *et. al.*, 1957). The authors suggested that the disease might have been introduced by war veterans from Korea. If this is the case, similar foci of infection may have developed in many other areas where suitable conditions for spreading of the intermediate hosts exist and where people habitually consume raw crabs and shrimps.

The toll in morbidity and mortality of paragonimiasis still remains undetermined, even in areas where this disease is known to be endemic. The number of clinical cases can only be estimated from the number reports appearing in the medical literature and this is, at best, a dubious estimate, since most of the patients do not seek medical care and, among those who do, many are diagnosed clinically as having other infections.

Of particular interest from the standpoint of public health is the frequent mistaken diagnosis of paragonimiasis for tuberculosis. This occurs primarily during routine X-ray screening tests for tuberculosis, but it also takes place in daily clinical practice. It was observed by Yokogawa that large numbers of patients with pulmonary paragonimiasis are hospitalized in sanitoriums or treated for tuberculosis at home. Our findings in Korea confirm Yokogawa's observations. Not infrequently, lobectomy was mistakenly performed on individuals suffering from lung fluke disease. A considerable percentage of patients who had been diagnosed as having tuberculosis and had been treated for that disease for several years, indeed were suffering from paragonimiasis. Cerebral paragonimiasis was frequently misdiagnosed as tuberculous meningitis.

To insure correct diagnosis of paragonimiasis in extra pulmonary infections as well as in early, mild, latent or treated cases, laboratory methods other than sputum or stool examinations are necessary. Radiological means of diagnosis (Ch'ien Mu-Han, 1955; Rose *et. al.*, 1952) are expensive and require a high degree of technical skill, especially where *P. westermani* cysts are not near the ventral surface of the lungs. The interpretation of chest films is necessarily complicated by the need to distinguish between tuberculosis and paragonimiasis. Therefore, it is important to have much more information than is now available about the diagnosis and prevalence of paragonimiasis, not only in order to assess properly the public health significance of

this disease in the Far East, but also to plan and interpret the results of tuberculosis control programs.

Because of these considerations, efforts of several investigators have been directed toward the development and evaluation of immunological methods (Hosokawa *et al.*, 1955; Yokogawa *et al.*, 1955, 1956; Chung *et al.*, 1955, 1956; Hunter *et al.*, 1958; Ando, 1910) for the diagnosis of paragonimiasis. In our laboratory, attempts were carried out to develop purified antigens which would give reliable results when used in the intradermal and complement fixation tests for paragonimiasis.

INTRADERMAL TESTS

Altogether, more than 3,000 intradermal tests for paragonimiasis were carried out in Korea by our laboratory in cooperation with the German Red Cross Hospital. The testing was generally extended to school children and village populations living in well defined geographic areas as well as to hospital and clinic patients. The results were interpreted qualitatively by the percentage of positive reactors and quantitatively by the size of the wheals 15 minutes after the infection. The places in which the team worked, the kind and size of the population tested and the results have been presented in Table 1.

In areas where paragonimiasis is prevalent, most people show a high degree of sensitivity to the skin testing antigen. A large percentage of them, in addition, have X-ray evidence of pulmonary infiltration. A certain percentage of these have *Paragonimus* eggs in the sputum. Where the infection is less prevalent, evidence of its existence may be limited to well circumscribed localities or to only a small group of people who have a history of consuming freshwater crabs from endemic areas.

Intradermal sensitivity to *Paragonimus* antigen is not, in itself, incontrovertible evidence of infection with *P. westermani*. Other trematodes are known to cross-react with *Paragonimus*, although the reactions of the homologous antigens generally are much greater. In some Korean areas where infection with *C. sinensis* is very prevalent, a relatively high proportion of the population reacted both to *Para-*

gonimus and to *Clonorchis* antigens. But the *Paragonimus* reactions, as a rule, were weak and present only in those persons giving strong dermal reactions to the *Clonorchis* antigens. On the other hand, only

TABLE 1 — *Cutaneous reactions to a purified P. westermani antigen in different locations in South Korea*

Geographic location	Type of population	Epidem. information	Number tested	Percent positive
Kanjon-Ri . .	School children & general population	On stream — fresh-water crabs	370	78
Sogi-Ri . . .	School children & general population	On stream — fresh-water crabs	502	49
Naedo-Ri. . .	School children & general population	On stream — fresh-water crabs	97	62
Chochun-Ri. .	General population	Off stream — sea crabs	100	4
Namwon-Ri. .	School children & general population	Off stream — sea crabs	200	11
Aewol-Ri. . .	School children & general population	Off stream — sea crabs	100	14
Un-Dong. . .	General population & school children	Off stream — fresh-water crabs <i>Clonorchis</i> endemicity	419	27
Tae-Djo . . .	School children	On river — heavy <i>Clonorchis</i> endemicity	356	6
Pusan — Red Cross Hospital	Tuberculosis patients	Kyung San Namdo province — heavy <i>Clonorchis</i> endemicity	138	19

occasional weak reactions to *Clonorchis* antigens were found among individuals who strongly reacted to *Paragonimus* in areas where paragonimiasis is endemic. Testing simultaneously with these trematode antigens could be expected to furnish strong presumptive

evidence of specific infections in areas where infection with *P. westermani* is considered dubious.

While often closely resembling pulmonary tuberculosis in its clinical and roentgenological appearances, paragonimiasis differs strikingly in its epidemiology, treatment and prognosis. The factor of primary significance in paragonimiasis is geography. Where man lives and what he eats, rather than with whom he lives, is of major importance in determining his chances of becoming infected with this parasite. Since there is no evidence that purified trematode antigens give cross-reactions with tuberculosis, if *Paragonimus* dermal sensitivity is observed in tuberculous persons, one has good reason to suspect that both infections may be present in the individual.

Histoplasmosis also closely resembles pulmonary tuberculosis and paragonimiasis, both clinically and roentgenologically. However, in general, infections with this fungus are not prevalent in those geographic areas where paragonimiasis is endemic. A number of cases of histoplasmosis have been diagnosed in Indonesia and a small proportion of reactors was found in the Philippines, Burma and Australia. Although only a limited amount of testing for histoplasmosis has been done in the Far East, the results available would tend to suggest the absence of it in those countries where studies have been carried out, such as Pakistan, India, Vietnam, Hongkong, Taiwan and Japan (Katayama *et al.*, 1953; Edwards and Kiaer, 1956).

SEROLOGICAL STUDIES

Serological studies are also of diagnostic value in paragonimiasis, particularly in active infections, and may help considerably in interpreting the significance of positive dermal reactors in epidemiologic studies (Ando, 1921; Chung *et al.*, 1956; Yokogawa and Awano, 1956; Sadun, 1957; Sadun *et al.*, 1958). As indicated in Table 2, in our experience, there was a close correlation between results of complement fixation, intradermal tests and sputum examinations. Only a small fraction of those individuals with proven active paragonimiasis gave negative complement fixation reactions. Conversely, none of the individuals with negative intradermal tests gave positive complement fixation reactions. Most of the sera from treated individuals who were

positive dermal reactors, even though no eggs could be recovered in the sputum (Group II), gave negative complement fixation reactions.

Studies on experimentally infected animals indicated that the complement fixation test becomes positive approximately 1 month following infection, whereas eggs in the sputum cannot be detected for at least 2 months. Because of this consideration, the complement fixation test is valuable in the detection of paragonimiasis in the early stages. Using a purified *Paragonimus* antigen, the complement fixation test of sera from individuals with clinically related conditions such as tuberculosis, histoplasmosis, coccidioidomycosis, sarcoid, actinomycosis and carcinoma of the lung were negative. Also, no cross-

TABLE 2 — *Comparison of results of complement fixation tests, intradermal tests and sputum examinations on the same individuals*

Group	Sputum exam.	ID test	Results of complement fixation test			
			Positive	Negative	Anticomp.	Total
I	Positive	Positive	72	8	6	86
II	Negative	Positive	6	14	1	21
III	Negative	Negative	0	51	4	55
Total. . .	—	—	78	73	11	162

reactions were observed with sera from individuals with trematode infections which are clinically distinguishable but taxonomically related to *Paragonimus* infection, such as clonorchiasis and schistosomiasis. By the use of this purified antigen, false positive reactions which had been reported previously with conditions such as syphilis, tuberculosis and leprosy were also not observed. The apparent specificity of this test, added to the consideration that negative results are obtained in treated individuals, even though roentgenological examination and intradermal tests are positive, make us regard the complement fixation test of great value from the standpoint of clinical practice as well as for a proper evaluation of the public health significance of paragonimiasis.

SUMMARY AND CONCLUSIONS

Paragonimiasis is associated with the consumption of uncooked fresh-water crabs, and constitutes an important public health problem in Asia, particularly in Korea, Japan and China. The prevalence of paragonimiasis still remains somewhat unknown, since most of the patients do not seek medical care and, among those who do, many are diagnosed clinically as having tuberculosis or other infections. *Paragonimus westermani* is known to be endemic in some geographic areas and absent in others, but for many parts of Asia still little information is available on whether or not paragonimiasis has to be reckoned with as a significant public health problem. From the data currently available, paragonimiasis would appear to be primarily a pulmonary disease, but the number of people with extra pulmonary paragonimiasis seems to be much greater than previously anticipated. Intradermal tests should be used widely and related to the radiological evidence of pulmonary infiltration, and to epidemiologic observations of the presence of infected fresh-water crabs. By confirming these observations with the results of sputum examinations and serological tests in few individuals, it should be possible to draw a much more complete picture of the distribution and public health significance of paragonimiasis in Asia than is available today. Of particular significance is the common mistaken diagnosis between paragonimiasis and tuberculosis. The social and economic impact of these mistaken diagnoses is obvious and need not be emphasized.

As it has been pointed out by Stoll (1943), in his interesting discussion of the changed viewpoint on helminthic diseases, long ago, a distinguished English helminthologist (Lieber, 1924) mentioned that «the intense activity with which research has been pursued during recent years has resulted in knowledge which, if properly applied, ought to insure the control and eventual eradication of the infestations of man with parasitic worms», but, he added, «of these questions, the medical officer of health is, as a rule, lamentably ignorant». Today, 34 years after this statement was published, the gap between scientific knowledge on paragonimiasis and the application of this knowledge in endemic areas has not, as yet, been bridged.

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THE *IN VITRO* ASCARICIDAL EFFECT OF THE LATEX
OF 26 SPECIES OF *FICUS* AS WELL AS *PAPAYA CARICA*
AND *SYNADENIUM GRANTII*

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The anthelmintic action of the latex of certain *Ficus* trees was known in South America as early as the 18th century. The latex most commonly used is obtained from certain species of *Ficus* trees, namely *F. glabrata* (*F. laurifolia*), which is locally named «leche de higuérón», and from *F. doliaria*, «leche de gamelleira». The effect on the worms was at first assumed to be due to the acid or corrosive properties of the latex and Bajon (1770) as well as Rodriguez de Maura (1866) pointed out that great care should be taken that the patient treated with the latex should be free from inflammation of the intestinal tract. The action of the latex against hookworms is disputed. Against *Trichocephalus* it is very effective and Calle and Uribe (1911) observed that no other drug was as effective against these worms as «leche de higuérón», treatment which could be repeated many times with no ill effects. Posada Berrio (1912) as well as Spritt (1921) and Caldwell and Caldwell (1929) obtained very good results with the drug against the worm. Hall and Foster (1918) and Caldwell and Caldwell (1929) found that the drug was also very efficient against *Ascaris*, but had no effect whatsoever against tapeworms.

The first chemical studies on the latex of *F. doliaria* demonstrated a glucoside named *doliarina* and a proteolytic enzyme termed *urostigma papayolin* (Peckholt, 1861) and the digestive action of the latex was demonstrated by Moncorvo (1881). In 1930 Robbins isolated the active principle from «leche de higuérón», which he termed *ficin* and which he found to be capable of digesting living *Ascaris* and hydrolysing coagulated egg white and casein at optimum pH between 5 and 8.5. He concluded that the principle is a proteolytic enzyme like papain of the tryptic type.

In 1934 Robbins and Lamson tested the enzymatic action of the sap from 15 species of the genus *Ficus* from Cuba and *F. carica* from Alabama and one unknown species of *Ficus* and from other 4 genera of the family *Moraceae* using gelatine and pig *Ascaris* as test objects. To 100 cm³ Ringer at 35°C containing living *Ascaris* they added 2 cm³ of the sap to be tested, using *F. glabrata* sap as a control which was found in 1-2 percent to digest *Ascaris* in 1.5-2 hours. The saps were collected in mid November. They concluded that the activity of all except 2 of these 16 species was essentially nil. The types of *Ficus* and *Moraceae* trees' saps which were tested were as follows:

Ficus aurea, *F. benghalensis*, *F. benjamina*, *F. brevifolia*, *F. car-pensis*, *F. crassinerva*, *F. elastica*, *F. glabrella*, *F. glamarata*, *F. nitida*, *F. nota*, *F. religiosa*, *F. spragnuena*, *F. vogelii*, *F. carica*, *F. glabrata*, *F. unknown*, *Atrocarpus foret*, *Mosimum alicastrum*, *Broussonetia papyrifera*, *Morus nigra*, *Higuerón hembra*, *Higuerón rojo hembra*, *Higuerón milagroso* and *Higuerón liso rojo*.

The two effective ones were *F. carica* and *F. glabrata*. The latex from the 4 genera of the family *Moraceae* showed an activity less than 1/5 that observed in *F. carica* collected at the same time. In further studies, Robbins (1935) observed that there was a marked seasonal variation in the amount of enzyme present per unit volume of sap of *F. carica*, the concentration being lowest in early summer. The latex of *F. laurifolia* and *F. doliaria* were found to be active *in vitro* as is that of *F. pumila* (Asenjo, 1939). A more refined product was prepared by Bliss which he termed *ficus protease*, which was tested *in vivo* and *in vitro* by Thomen (1938) and found to exert a high proteolytic action on coagulated egg albumin as well as on pig

Ascaris and to be a highly efficient anthelmintic against *Trichocephalus* of dogs. Walti (1938) obtained a crystalline form of ficin with the same anthelmintic properties as the original latex when tested on living *Ascaris* and gelatine. The enzyme was found by Cornatrez (1942) not to be destroyed by the digestive properties of the dog.

Faust and Thomen (1941) found that the latex preserved with 0.1-1 percent sodium benzoate was less effective than unpreserved refrigerated latex.

The aim of the present study was to examine the saps collected from different *Ficus* trees grown in some Cairo gardens as well as the local fig trees to ascertain their anthelmintic properties. The study was made by testing the action of the sap on living *Ascaris* worms *in vitro*. The saps were collected from trees grown in the gardens of the Faculties of Agriculture of the Ein-Shams and Cairo Universities, Ein-Shams University Faculty of Science, Orman Gardens and Zoo Gardens in Giza. *Papaya carica* latex was collected from young trees and immature fruits during the month of December 1956, while that of *F. carica* was obtained during October 1957, and that of the rest during the period from March to June 1957. The collected saps were kept without preservation in a refrigerator at 5°C and the tests were performed with each type of latex within 10 days after collection of the material.

Living cattle and pig *Ascaris* were collected from Cairo abattoir from animals immediately after slaughtering and kept in Ringer solution at 37°C. Each batch of worms could be kept in a good state for at least 48 hours without losing any of their vitality, during which time testing of the material would be performed.

The species of *Ficus* and other trees whose sap was tested included the following:

<i>F. sychamoris</i>	<i>F. villosa</i>
<i>F. benamina</i>	<i>F. rubiginosa</i>
<i>F. trijuga</i>	<i>F. pseudosychamoris</i>
<i>F. macrophylla</i>	<i>F. benghalensis</i>
<i>F. glomerata</i>	<i>F. infectoria</i>
<i>F. roxburghi</i>	<i>F. mysorensis</i>
<i>F. religiosa</i>	<i>F. salicifolia</i>

<i>F. spragnuena</i>	<i>F. glaberrima</i>
<i>F. elastica</i>	<i>F. tseila</i>
<i>F. retusa</i>	<i>F. eriobotryoides</i>
<i>F. carica</i>	<i>F. platypoda</i>
<i>F. nitida</i>	<i>F. asperima</i>
<i>F. cunninghami</i>	<i>Papaya carica</i>
<i>F. vogelli</i>	<i>Synadenium grantii</i>

Trials to obtain latex from another 3 species of *Ficus*, namely *F. hispida*, *F. gibbosa* and *F. vasta*, were made, but the material collected was not enough to allow study due to its scantiness and stickiness. The anthelmintic properties of *Papaya carica* were reported by Hassler (1928) and Asenjo (1941), the effect being due to the presence of the enzyme papain, and sap from this tree was included in the present study for comparison with that from the other trees.

Living *Ascaris* worms were put immersed in beakers containing each about 50 cm³ of the sap to be tested and incubated at 37°C. Control living *Ascaris* were put in beakers containing Ringer solution at the same temperature and incubated at the same time. The worms were examined at different times for signs of action by the sap as loss of luster of the cuticle, flaccidity, and death of them as indicated by complete flaccidity, blistering of the cuticle and fragmentation of the worm.

The results are forwarded in table.

Crude saps of *F. cunninghami*, *F. vogelli*, *F. macrophylla*, *F. glaberrima*, *F. trijuga*, *F. glomerata*, *F. eriobotryoides*, *F. mysorensis*, (*F. laccifera*), *F. infectoria*, *F. sychamoris*, *F. benghalensis*, *F. nitida*, as well as *F. spagnuena*, 50 percent, *F. retusa* 50 percent, *F. religiosa* 50 percent, *F. elastica* 30 percent, *F. roxburghi* 25 percent, *F. asperima* 20 percent, *F. tseila* 20 percent, *F. villosa* 10 percent, *F. salicifolia* 10 percent, *F. rubiginosa* 5 percent and *F. platypoda* and *F. benjamina* 1 percent each; in Ringer solution did not affect the worms apart from slight flaccidity after 24 hours in the case of *F. villosa* and *F. platypoda* and even in the case of *F. religiosa* the worms were observed to be more active. Only those of *Papaya carica*, *F. carica* and *F. pseudosychamoris* were found to kill and digest *Ascaris*.

The lowest dilution of *P. carica* to achieve this effect within 2 hours was 0.15 percent. Latex from this tree has been found to have anthelmintic properties by Hassler (1929) and Asenjo (1941) and the action is known to be due to the latex content of papain, an enzyme closely similar to ficin. Extracts from *F. carica* could kill and digest *Ascaris* within 2 hours in a dilution of 1.6 percent. Latex from these two trees was obtained in the month of December in the case of the former tree and November in the case of the latter. Sap from *F. pseudosychamoris* could kill and digest *Ascaris* within 2 hours up to a dilution of 6.25 percent, although further dilutions, up to that of 0.4 percent, could bring about this result within 24 hours. Sap from this tree was collected during the month of June and it is probable that if it was obtained during the winter, when the sap is more concentrated, higher dilutions would have been found more effective.

Sap from *Synadenium grantii*, a kind of hook, in a dilution of 80 percent in Ringer solution had no effect on the worms during the first 4 hours, although the worms were found dead 24 hours after being immersed in the sap which was found glued around them, the effect most probably being mechanical in nature.

F. carica is the common fig tree in Egypt and is locally called «Teen Barshoumy» and grows abundantly in sandy areas like Dekhela and Marsa Matrouh, while *F. pseudosychamoris* is locally called «Teen Barry» (wild fig) and is found in areas of Sinai desert where water is abundant. *Papaya carica* is imported from warmer countries although it can be grown with ease in Egypt. *In vivo* trials with these saps are essential before assessing their value as anthelmintics.

CONCLUSION

Latex from 26 species of *Ficus* collected from different trees grown in some Cairo gardens as well as from the stem and fruit of *Papaya carica* and *Synadenium grantii* were tested in different concentrations for their action on living pig and bovine *Ascaris* worms. Only those obtained from *F. carica*, *F. pseudosychamoris* and *P. carica* were found effective as indicated by digestion and death of the worms. The lowest concentration that could kill and digest the worms within

2 hours was 0.15 percent in the case of *P. carica*, 1.6 percent in the case of *F. carica* and 6.5 percent in the case of *F. pseudosydamoris*. Sap from *Synadenium grantii* killed *Ascaris* within 24 hours probably by mechanical action, no digestive action being observed.

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EPIDEMIOLOGIC CLASSIFICATION OF THE PRINCIPAL HUMAN HELMINTHIASES

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It is customary to classify helminthiases on the basis of systematic arrangement of their agents. This seems to be quite logical when we regard and characterize the helminthiasis agents as a specific group of the animal world.

However, to the applied biological sciences, medicine and veterinary among them, an epidemiologic classification of helminthiases and their agents is also of importance, since the measures of helminthiases control are first and foremost based on the knowledge of helminthiases epidemiology. But in order to understand the latter correctly, one must possess the knowledge of the main stages in the development of the helminths.

The aim of this paper is to give a scientific epidemiologic classification of helminthiases and their agents.

K. I. Skryabin and R. S. Schultz (1931) suggested that, depending on whether the helminthiases agents have the intermediate host or not, all helminthiases should be divided into geohelminthiases, caused by the geohelminths, which develop without the intermediate host, and biohelminthiases, caused by the biohelminths, which complete their development in one or more hosts.

To extend this classification of geo- and biohelminths, we suggest that both geo- and biohelminths should be subdivided into groups characterized by different requirements of the early helminth stages to their environment outside the host organism.

Finally, it seems logical to further subdivide these groups into smaller subgroups proceeding from the differences in the mechanism of infection of the final host.

In the accompanying diagram we have summarized our epidemiologic classification of the principal human helminthiases, which we propose, basing it on the above-mentioned three factors, viz. (1) presence or absence of the intermediate host, (2) relation of the early helminth stages to their environment outside the host organism, and (3) mechanism of infection of the final host.

Geohelminthiases are characterized by the fact that their agents develop without the intermediate host, and also by the fact that their early stages — the eggs, and in some cases also the larvae that had emerged from the egg-shells — can develop only in the environment outside the host organism.

Leaving for the time being the question of *Enterobius vermicularis*, which, if compared with the rest of the geohelminths, possesses some peculiar features, we shall dwell now on the characteristics of the rest of the geohelminths.

Group I. — Man acquires infection through swallowing helminth eggs fully developed in natural conditions. Here belong *Ascaris lumbricoides* and *Trichocephalus trichiurus*.

Group II. — Man acquires infection through active penetration through the skin of the geohelminth larvae which had reached the invasion stage in the soil. Here belong *Ancylostoma duodenale*, *Necator americanus*, *Strongyloides stercoralis*.

As far as *Enterobius vermicularis* is concerned, its mechanism of infection is analogous to that of *A. lumbricoides* and *Trichocephalus trichiurus*. *Enterobius vermicularis* differs from the rest of the geohelminths in that the conditions favouring its development are not the microclimate of the soil, as is the case with the other helminths, but the microclimate of the surface of the human body underneath the clothes or while in bed (34-36°C).

Due to this peculiarity of *Enterobius vermicularis*, it is placed apart from the rest of the geohelminths in our diagram.

Biohelminthiases are primarily divided into two groups.

Group I. — Biohelminthiases whose agents must necessarily change hosts. Here belong all biohelminthiases except hymenolepidosis caused by *Hymenolepis nana*.

Group II. — Biohelminthiases whose agents do not necessarily change hosts. To be more precise, at the present stage of their phylogenetic development they do not generally require a change of host. Here belongs hymenolepidosis caused by *Hymenolepis nana*.

As is known, *H. nana* develops without the intermediate host. But the part of the intermediate host of this cestode is played by the villi of the host's small intestine, where the development of the helminth's larvae stages takes place. As was shown by the experiments made in France by Joyeux and Bear (1929), this parasite's larvae can develop in insects destructive to flour (*Tenebrio molitor* and *Tenebrio obscurus*). The work of Bacigalupo (1928) in Argentina showed that in that country, besides the above-mentioned insects, the part of the intermediate host can be played by some species of the flea. In Argentina they succeeded not only in effecting experimental infection, as had been done in France, but in discovering in natural conditions intermediate hosts spontaneously infected by cysticercoids.

Feeding mice on these intermediate hosts caused the invasion of the mice with adult specimens of *Hymenolepis fraterna*, which, in our opinion, is identical with *Hymenolepis nana*.

All these experiments and observations are a confirmation of the phylogenetic connexion between *H. nana* and the biohelminths and of the correctness of its inclusion into the biohelminth group, as is done in our diagram.

Thus, in the geohelminth group, a special place is reserved for *E. vermicularis*; and in the biohelminth group, for *H. nana*. At the same time, both these helminths have very much in common as regards their epidemiology, since the infection with both the helminths takes place in the immediate environment of man and can be acquired either directly after the eggs get into the environment — in the case of *H. nana* — or almost immediately (4 to 6 hours after the eggs get into the environment) — in the case of *E. vermicularis*. This likeness between the two helminths is shown in the diagram.

According to the relation of the early stages of the other biohelminths to their environment outside the host organism, they can be divided into three groups, each of which, in turn, can be subdivided into two subgroups.

Group I. — Here belong the biohelminths whose early stages do not require the environment outside the host organism.

(1) The first subgroup includes biohelminths representative of the suborder *Filariata* Skryabin, 1915, whose infection of the final host (man for some species, and animals) is acquired through the bite by the intermediate host (various species of mosquitoes and gadflies). In their turn, the intermediate hosts contract infection from the final hosts by sucking their blood containing the larvae of these biohelminths (microfilariae).

Here belong the species of the genera *Wuchereria*, *Loa*, *Onchocerca* and some other species.

(2) In the second subgroup *Trichinella spiralis* must be included, owing to its manner of infection (the host ingests meat containing its larvae) and some peculiarities in its development.

Group II.—The early stages of the biohelminths belonging to the second group need the environment outside the host organism only for the contact with, and infection of, the intermediate host. No development of the larval stages outside the host organism takes place.

(1) The first subgroup of the biohelminths belonging to group II is characterized by the following mechanism of infection of the final host (man and certain animals): the biohelminth larvae are ingested by the final host when eating the organs of the invaded intermediate host or when accidentally swallowing the intermediate host containing biohelminth larvae if these latter appear invisible to the naked eye.

Here belong the cestodes *Taeniarhynchus saginatus*, *Taenia solium* and the nematode *Drancunculus medinensis*.

(2) The infection of the final host, including man, is acquired through active penetration through his skin and into his tissues of the larvae which had developed in the intermediate host till they reached the invasion stage and had migrated from that organism into the water (the species of the genus *Schistosoma*).

Group III.—Like the geohelminths, the biohelminths belonging to group III need the environment outside the host organism for the embryonic development of their early stages. They differ from the geohelminths in that their larvae, which develop within the eggs and then outside the egg-shells, are still incapable of infecting the final host as the geohelminths are. The larvae's development must continue in the intermediate host and, in the case of some biohelminths, their development continues in the second intermediate host.

(1) The biohelminths belonging to the first subgroup of this group require a change of two hosts. The larvae that had completed their development in the intermediate host leave the latter, become encysted, and attach themselves to some plants. Infection of the final host is acquired by eating raw plants and drinking water containing encysted larvae derived from infested plants.

Fasciolopsis buski and *Fasciola hepatica* develop in this manner.

(2) The development of the biohelminths belonging to the second subgroup of group III proceeds with a change of three hosts. The final host (man and a number of carnivorous domestic and wild animals) acquires infection either by eating the flesh of the second intermediate host or by ingesting the whole host. Here belong human parasites of the genus *Diphyllbothrium* and many trematodes whose most important species are given in our diagram.

GENERAL DISCUSSION

DR. N. SWELLENGREBEL (Netherlands) — Dr. S. asks whether *E. multilocularis* in *Microtus* — and other natural intermediate hosts — is a more natural parasite than it is in man (in the shape of *E. alveolaris*, of ill repute).

DR. R. RAUSCH (U.S.A.) — Voles tolerate natural infections with *E. multilocularis* very well. Most infections involve few eggs, and if the voles are not killed by predation they are usually able to tolerate the infection for their entire life, which does not often exceed a year's time.

Very great numbers of scolices are produced in voles, in old infections there are about 300 larvae per milligram of larval tissue. The cycle naturally involves voles and foxes. In man it is purely accidental.

DR. A. NEGhme (Chile) — *To paper of Dr. Rausch:* I congratulate myself for being acting chairman of this meeting, when such an important paper like that of Dr. Rausch have been delivered. Thank you very much for your very fine piece of work. *To paper of Dr. Sadun:* Did you look for delayed type of cutaneous reactions on your paragonimiasis patients?

DR. E. SADUN (U.S.A.) — *Answering to Dr. Neghme's question:* We studied the time of appearance and persistence of dermal reaction in large numbers of persons living on endemic areas. As a rule, the size of the ensuing, what increased during the first 15-20 minutes following antigenic introduction. It then decreased and in most persons it disappeared by 60 minutes following inoculation. Only in rare cases was there any persistence of reaction after 24 hours.

DR. GILBERT F. OTTO (U.S.A.) — Until the work of Dr. Rausch in Alaska and that of Dr. Vogel in Germany, we were accustomed to

think of the epidemiology of all human echinococcosis in terms of the ecology of *Echinococcus granulosus*. It was very disconcerting then to find in Alaska very high serological and intradermal rates with little or no evidence of disease. Such rates were found both among the Eskimos and among United States nationals on St. Lawrence Island. The picture is not yet completely clear, but three alternatives are suggested: (1) that with the high exposure rate many echinococcus eggs are ingested and transient, but abortive metabolism sensitized large portions of the population, (2) that some unexplained non-specific worm reaction is involved, or (3) that extracts of host tissue in the antigen are producing the reactions. It seems to me probable that the first of these alternatives is the most significant. However, regardless of the ultimate explanation, we were unable to appreciate the nature of the problem before the careful taxonomic and ecological studies of Rausch in Alaska and Vogel in Germany.

PAPERS OFFERED AS FREE COMMUNICATIONS TO THE
SUBSECTION 3—OTHER INFECTIONS BUT NOT READ
DURING THE MEETINGS DUE TO THE ABSENCE OF
THE AUTHORS

ASCARIASIS AND CHRONIC INTESTINAL AMEBIASIS

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One of the many problems concerning intestinal amebiasis is its stubbornly recurrent nature, which is characteristic of this disease.

In many cases it is possible to find by careful history the precipitating causes of recurrences. These may include gross dietary indiscretions with digestive upsets, overheating, considerable physical strain or bacterial enteric infection.

In two of our cases the provoking factor appeared to be overindulgence in alcohol, in a third case, drinking cold water after overheating.

In spite of our most careful analysis of the patients' condition preceding illness, we often fail to find any change in the patients' regime to explain recurrence.

In our patients we observed one important correlation: 137 individuals, i.e., 88,3 percent, suffered simultaneously from enteric amebiasis and ascariasis. In most cases the diagnosis ascariasis was made by finding ova in the stool; very often, however, the patients mention spontaneous discharge of intestinal worms with the stools.

At this juncture it may be useful to review briefly the adverse effects on the patient of the presence of ova and worms. *Ascarides lumbricoides* live, as a rule, in the small intestine, but they also often

occur in the large intestine. Both worms and ova may cause illness in several ways: mechanically by releasing toxins, by causing allergy or malnutrition.

The most dramatic are their mechanical effects, of which there are many case reports. They may cause ileus verminosus, biliary colic, pancreatitis, appendicitis, suffocation caused by inhaled worm, etc. When considering the relationship of ascariasis to amebiasis, the most important effects of the former are local, mechanical disturbances of the intestinal mucosa. Even when the worms move freely in the intestinal lumen, they may damage mucous membrane with their mouths (Skorpil). The mechanical along with toxic effects may result in perforation of the intestine followed by peritonitis. No less important is the fact that these factors influence the interoceptors of the intestinal tract, which results in affecting the intestinal motility, being probably one of the main causes for intestinal colic; these, of course, frequently occur with ascariasis.

That ascarides have a direct toxic effect on the patient now seems beyond doubt, despite some disagreement concerning its pathogenesis. The Japanese have isolated a toxin-ascaron. An injection of 0.1 mg of this toxin was lethal for an adult horse. The extracts from intestinal worms are toxic and cause hemolysis. Skorpil mentioned that, in anatomical preparation of ascaris, a tiny drop of its tissue fluid causes irritation or inflammation of the skin or mucous membrane. 2 ml of this tissue fluid kill a rabbit within 10 minutes.

Toxic effects of ascaris are probably responsible for clinical symptoms distant from the area where worms are localized. Thus, epileptiform convulsions are described, which disappeared following antihelminthic therapy. Meningeal symptoms have also been noted, paralysis of n. III, IV, VII, XII. There has even been reported a case of paraplegia in a 7 year old child, where function was completely restored following the expulsion of ascarides. Chaufford describes the so called typholumbricosis with fever and diarrhea.

In certain cases, ascaris or its toxin seems to act as an allergen. In cases of ascariasis, authors described bronchial asthma, urticaria, itching in the nose, conjunctivitis. Goldschmiedt describes many allergic manifestations in medical and scientific personnel working in contact with ascaris, occasionally severe.

Naturally the noxious effects of ascarides — toxic, mechanical, malnutritive, allergic — are aggravated in the presence of chronic changes caused by intestinal amebiasis.

In the light of these considerations attention was turned to antihelminthic therapy in those cases of chronic amebiasis where, after antiamebic therapy, stools improved in quality and quantity, the general condition improved, but where abdominal colic, tenesmus, anorexia and general irritability persisted. These complaints usually vanished after expulsion of ascaris, and the quality of the stools got sometimes improved as well.

Following encouraging preliminary results, with such combined therapy, treatment of ascariasis was made routine in the treatment of intestinal amebiasis.

In patients whose conditions permitted, treatment for ascariasis was started first, and, after the parasites were expelled, antiamebic therapy was begun. The patients who on admission were in serious condition with frequent diarrhea and signs of dehydration were first given general and antihelminthic therapy. This was our procedure with the 82 patients suffering from ascariasis and intestinal amebiasis. In the majority of our patients we gave santonin, only in a few cases we used piperazine and hexylresorcinol.

In an attempt to determine whether ascarides may not be carriers of amebic cysts, the tissue fluids preparations of the worms were examined for cysts. Results were entirely negative: no cysts were found in ascaris.

We gave treatment for ascarides on all our patients suffering from amebiasis, in whom ascarides were also found, despite our certainty that any patient returning to his original surroundings would be exposed to infestation with ascarides. It was hoped that before reinfestation was well established and the larvae matured, there was a sufficient interval for the mucous membrane to heal.

Two cases were of particular interest:

1 — A 46 year old woman admitted with diagnosis: intestinal amebiasis, ascariasis. Biliary colic complicated intestinal amebiasis and simulated perforation with peritonitis. The colic was caused by obstruction of the bile duct by a worm.

2 — An 11 year old boy suffering from amebiasis and operated upon acute appendicitis had an abscess the size of the walnut found

in the wound after laparatomy. In the microscopic examination of the pus there were ascarides ova. Neither amebae nor their cysts were found.

SUMMARY

The frequent coincidence between ascariasis and chronic intestinal amebiasis has been described. Worms and their ova cause the irritation of the intestinal mucous membrane in a mechanical, toxic and allergic way. This affects intestinal interoceptors which results in disturbances of innervation of the intestinal musculature. In addition, the worms cause constitutional disease by consuming part of the hosts' nutrients, and by a general toxic influence. No cysts of amebae were found in the tissue fluid of ascaris. For successful treatment of amebiasis, simultaneous therapy for ascariasis appears imperative despite the probability of reinfestation with ascariasis. The author quotes a case where biliary colic was caused by a worm entering the bile duct. Ascaris brought out with vomiting showed bile colouring in the part that entered the bile ducts.

Next, a case is described where an ascaroid subcutaneous abscess was formed in the wound after laparatomy.

ESTUDO ESTATÍSTICO SOBRE A VARIAÇÃO DA FREQUÊNCIA DA ANCILOSTOMÍASE COM O SEXO E A IDADE

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Damos primeiramente a conhecer os resultados da pesquisa de helmintes e ovos destes nas fezes de 918 indivíduos naturais de Cabo Verde, residentes na ilha de S. Vicente e acabados de regressar das ilhas de S. Tomé e do Príncipe, onde foram trabalhadores de roças durante cerca de três anos. S. Tomé e Príncipe são duas ilhas portuguesas muito agrícolas situadas junto do equador.

QUADRO 1 — *Número e percentagem de indivíduos parasitados, por espécies de parasitas*

Parasitoses	Número	Percentagem
Nula	182	19,83
Por <i>Ancylostomidae</i>	172	18,74
Por <i>Trichocephalus dispar</i>	32	3,48
Por <i>Ascaris lumbricoides</i>	37	4,03
Por <i>Ancylostomidae</i> + <i>Trichocephalus</i>	175	19,07
Por <i>Ancylostomidae</i> + <i>Ascaris</i>	86	9,37
Por <i>Ascaris</i> + <i>Trichocephalus</i>	27	2,94
Por <i>Ancylostomidae</i> + <i>Trichocephalus</i> + <i>Ascaris</i>	197	21,46
Por <i>Ancylostomidae</i> + <i>Hymenolepis nana</i>	2	0,22
Por <i>Trichocephalus</i> + <i>Hymenolepis nana</i>	1	0,10
Por <i>Ancylostomidae</i> + <i>Trichocephalus</i> + <i>Hymenolepis</i>	4	0,43
Por <i>Anc.</i> + <i>Asc.</i> + <i>Tric.</i> + <i>Hymenolepis nana</i>	3	0,33
Totais	918	100,00

A percentagem geral de indivíduos portadores de ancilostomíase eleva-se a 69,6. É de crer que todos eles foram infestados em S. Tomé e Príncipe, porque em S. Vicente não existe endêmicamente esta helmintíase.

O nosso estudo estatístico refere-se à variação da frequência da doença com o sexo e a idade.

1 — ANCILOSTOMÍASE E SEXO.

Foi encontrada a seguinte distribuição por sexos:

QUADRO 2 — *Número e percentagem de homens e mulheres parasitados*

	Homens		Mulheres		Total
	Número	Percentagem	Número	Percentagem	Número
Com ancilostomíase	271	42,42	368	57,58	639
Sem ancilostomíase	116	41,58	163	58,42	279
Totais	387	—	531	—	918

Se compararmos os números de homens e mulheres pelo teste do χ^2 , obteremos $\chi^2=0,05$, com 1 grau de liberdade. Portanto, resultado não significativo (valor das tábuas 6,64, no limiar de 1 por cento), como deixava prever o quadro das percentagens.

2 — ANCILOSTOMÍASE E IDADE.

a) *Nas mulheres.*

QUADRO 3 — Número e percentagem de mulheres parasitadas

	Idades										Total
	0-9 anos		10-19 anos		20-29 anos		30-39 anos		+ 40 anos		
	Número	Porcentagem	Número	Porcentagem	Número	Porcentagem	Número	Porcentagem	Número	Porcentagem	Número
Com ancilostomíase . .	31	28,44	50	79,68	162	83,55	71	77,17	54	75,00	368
Sem ancilostomíase . .	78	71,56	14	20,32	32	16,45	21	22,83	18	25,00	163
Totais . .	109	—	64	—	194	—	92	—	72	—	531

Para o cálculo do χ^2 temos:

Frequência observada f	Frequência teórica f_1	$(f - f_1)$	d_1^2	$\frac{d_1^2}{f_1}$
31	75,53	44,53	1 983,544 3	26,259 2
50	44,35	5,65	31,899 9	0,719 2
162	134,44	27,56	759,443 3	5,648 8
71	63,75	7,25	52,475 5	0,823 0
54	49,89	4,11	16,842 8	0,337 5
78	33,46	44,54	1 983,544 3	59,275 7
14	19,64	5,64	31,899 9	1,623 5
32	59,55	27,55	759,443 3	12,751 3
21	28,24	7,24	52,475 5	1,857 9
18	22,10	4,10	16,842 8	0,761 9

$$v = 4$$

$$\chi^2 = 110,038 0$$

É significativo no nível de 1 por cento.

b) Nos homens.

QUADRO 4 — Número e percentagem de homens parasitados

	Idades										Total
	0-9 anos		10-19 anos		20-29 anos		30-39 anos		+ 40 anos		
	Número	Percentagem	Número	Percentagem	Número	Percentagem	Número	Percentagem	Número	Percentagem	Número
Com ancilostomíase . .	44	36,66	30	83,33	130	86,09	34	89,47	33	78,57	271
Sem ancilostomíase . .	76	63,34	6	16,67	21	13,91	4	10,53	9	—	116
Totais . .	120	—	36	—	151	—	38	—	42	—	387

Como há algumas classes de valor muito baixo, aplicámos no cálculo do χ^2 a correcção de Yates.

Frequência observada f	Frequência teórica f_1	$(f - f_1)$	$d_1 = (f - f_1) - \frac{1}{2}$	d_1^2	$\frac{d_1^2}{f_1}$
44	84,0	40,00	39,5	1 560,25	18,574 4
30	25,2	4,80	4,3	18,49	0,733 7
130	105,7	24,30	23,8	566,44	5,358 9
34	26,6	7,40	6,9	47,61	1,789 8
33	29,4	3,60	3,1	9,61	0,326 8
76	36,0	40,00	39,5	1 560,25	43,340 2
6	10,8	4,80	4,3	18,49	1,712 0
21	45,3	24,30	23,8	566,44	12,504 1
4	11,4	7,40	6,9	47,61	4,176 3
9	12,6	3,60	3,1	9,61	0,762 6

$$v = 1$$

$$\chi^2 = 89,278$$

É igualmente significativo no nível de 1 por cento.

c) Nas mulheres e homens em conjunto.

QUADRO 5 — Número e percentagem de homens e mulheres parasitados

	Idades										Total
	0-9 anos		10-19 anos		20-29 anos		30-39 anos		+ 40 anos		
	Número	Porcentagem	Número	Porcentagem	Número	Porcentagem	Número	Porcentagem	Número	Porcentagem	Número
Com ancilostomíase . .	75	32,75	80	80,0	292	84,63	105	80,76	87	76,31	639
Sem ancilostomíase . .	154	67,25	20	20,0	53	15,37	25	19,24	27	23,69	279
Totais . .	229	—	100	—	345	—	130	—	114	—	918

O cálculo do χ^2 dá-nos:

Frequência observada f	Frequência teórica f_1	$(f - f_1)$	$\frac{d^2}{f_1}$	$\frac{d^2}{f_1}$
75	159,38	84,38	7 119,984 4	44,672 9
80	69,60	10,40	108,160 0	1,554 0
292	240,12	51,88	2 691,534 0	11,209 0
105	90,48	14,52	210,830 4	2,330 1
87	79,34	7,66	58,675 6	0,739 5
154	69,62	84,38	7 119,984 4	102,269 2
20	30,40	10,40	108,160 0	3,557 8
53	104,88	51,88	2 691,534 0	25,663 0
25	39,52	14,52	210,830 4	5,334 7
27	34,66	7,66	58,675 6	1,692 8

$$\nu = 4$$

$$\chi^2 = 199,023 0$$

Altamente significativo.

CONCLUSÕES

1.^a A ancilostomíase distribui-se uniformemente nos dois sexos.

2.^a A mesma helmintíase é fraca nos primeiros anos de vida, aumenta bruscamente na adolescência e atinge o máximo na idade adulta, decrescendo depois dos 40 anos, isto talvez sob o efeito duma mudança de terreno (imunidade progressivamente adquirida).

Esta diferença de comportamento pode ser explicada por considerações de ordem epidemiológica: a ancilostomíase, de contaminação activa e transcutânea, atinge principalmente os indivíduos em plena actividade (adolescentes e adultos) trabalhando em terreno infestado.

Estas conclusões que a análise estatística permite tirar da população que estudámos devem poder receber aplicações de ordem profiláctica.

GNATHOSTOMIASIS IN JAPAN

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The disease is widely distributed in Asia, the most densely infested area being Thailand. On the other hand, in our country very little medical attention had been paid to the disease till the end of World War II, when in the northern part of Kyushu many inhabitants suffered from the disease. Since then clinicians began to pay attention to the disease and reported many interesting cases not only from Kyushu, but also from Shikoku and Honshu. The disease seems to be extending gradually.

The causative agent of the disease is called *Gnathostoma*. To the present, 18 species of the genus have been recorded in the literature, of which, however, 7 species seem to be distinct, namely *G. spinigerum*, *hispidum*, *turgidum*, *americanum*, *doloresi*, *nipponicum* and *procyonis*. In Japan there are 3 species, *spinigerum*, *doloresi* and *nipponicum*, of which, from the medical point of view, *spinigerum* is the most important.

The conspicuous clinical symptom of the disease is presented as cutaneous changes, of which migrating intermittent edema is usual and creeping eruption is rare form of the disease in Japan. Uncommon cases were also reported, in which the worm entered the cranial cavity, the eyeball, the lung, the kidney, the tongue, etc. As the cause of edema we regard not only the inflammatory and allergic reaction of the tissue, but also the toxic or enzymatic reaction: we found from the extract of the worm hyaluronidase and acetylcholine-like substance. Prior to the appearance of these symptoms, the disturbance of the liver function is recognized not only in experimental animals, but also

in human patients. This is due to the migrating character of the worm that they penetrate the gastric wall of the host and enter its liver.

A convenient and reliable means of clinical diagnosis for the disease is the skin-test. We inject intracutaneously 0.05 cm³ of a 50,000-fold saline solution of the antigen, prepared from the whole body of imagines or larvae of *Gnathostoma*, and 15 minutes later we judge by the diameter of the papule and the surrounding red areola. The positive reaction of the test is much more remarkable by injecting the protein fraction of the worm body than its lipoid or polysaccharide fraction, and the reaction is more conspicuous by injecting the extract of the esophagus than that of the other part of the worm body. For practical purpose of the skin-test, however, these troublesome handlings are not necessary.

As for treatment, there is not yet any effective medicine available; surgical removal of the worm from the cutaneous lesions is the only and the best method of treatment at present. It is interesting that in rare cases the worm spontaneously creeps out of the patient. Although there are many patients in Japan, only 14 cases were reported up to the present from which the causative worms were found. All the worms obtained were identified as *G. spinigerum*, 3 being larval and 11 being adult forms, 6 males and 4 females. Another worm was undecided because of the broken body.

Among the vectors of human gnathostomiasis, two species of freshwater fish, *Ophicephalus argus* and *O. tadianus*, are the most important. The former is widely distributed in Japan, but the latter is localized in the central part of Honshu. Japanese people like to eat their flesh in raw condition or after washing it with water. Moreover, some patients were infected the disease after eating the raw flesh of other fresh water fish or raw chicken.

Life history of *G. spinigerum* Owen, 1836: In Japan cats and dogs are naturally infected with the adult nematode. Of 51 cats examined in Fukuoka and Saga Prefectures, 14 (27.5 percent) proved to harbour 48 mature worms (28 females, 20 males) in their gastric walls and 21 immature ones principally in their livers, the maximum number of worms per host amounting to 22. Of 643 dogs examined, 26 (4 percent) were found to be infected with 41 worms, including 18 females, 13 males and 10 immature ones; the maximum number of worms per host was only 5 in these cases. On the other hand, tigers and leopards

imported from Thailand and Malay were found to be infected with the nematode by stool examination.

As the first intermediate hosts of the worm the following 4 species of cyclops were experimentally decided: *Mesocyclops leuckarti*, *Eucyclops serrulatus*, *Cyclops strenuus* and *C. vicinus*.

The second intermediate hosts of the worm are very widely distributed among pisces, amphibia, reptilia, aves and mammalia, because they become infected with the larval gnathostome either through infected first intermediate hosts — «primary infection» — or through infected other second intermediate hosts — «secondary infection». Almost all the larvae are found encysted in the muscles of these animals. Up to the present the following were proved to be naturally infected with the larvae: *Ophicephalus argus* and *tadius* (snake headed mullet), *Parasilurus asotus* (catfish), *Misgurnus anguillicaudatus* (loach), *Mogurnda obscura* (eleotrioid fish), *Anguilla japonica* (eel), *Acanthogobius hasta* (goby), *Cyprinus auratus* (crucian carp), *Rana nigromaculata* (frog), *R. catesbiana* (bullfrog), *Elaphe quadrivirgata* (striped snake), *Natrix tigrina tigrina* (grass snake), *Dinodon orientale* (snake), *Nycticorax nycticorax* (night heron), *Ardea cinerea jouyi* (eastern grey heron), *Egretta garzetta garzetta* (little egret), *Podiceps ruficollis poggei* (Chinese little grebe), *Pica pica japonica* (Korean magpie), *Rallus aquaticus indicus* (eastern water rail), *Alcedo atthis bengalensis* (common Indian kingfisher), *Scolopax rusticola* (woodcock), *Strix uralensis fuscescens* (Kiusiu Ural owl), *Corvus corone corone* (carrion crow), *C. coronoides hondoensis* (Hondo jungle crow), *Anas platyrhynchos domestica* (domestic duck), *A. crecca crecca* (teal), *Milvus migrans lineatus* (black eared kite), *Pandion haliaetus haliaetus* (osprey), *Accipiter gentilis fujiyamae* (goshawk), *Gallus gallus domesticus* (chicken), *Mergus serrator* (merganser) and *Mustela sibirica itatsi* (Japanese weasel).

Of these hosts naturally infected, *Ophicephalus argus* and *tadius* are, as stated above, the most important vectors of human gnathostomiasis in Japan. Accordingly, these fish, especially the former, have been investigated everywhere in our country. In Fukuoka Prefecture, Kyushu, we examined 450 last year, and found 444 (99 percent) harboured the larval gnathostome, its maximum number per host amounting to 330. On the other hand, the night heron, *N. nycticorax*,

has been intensely examined in each endemic area; in Fukuoka and Saga Prefectures the heron was found to be very heavily infected with the larvae, its percentage amounting to almost 100 percent. The localities where the larvae were so far found in the flesh of *O. argus* or *tadianus* are as follows: Fukuoka, Saga, Nagasaki and Kumamoto in Kyushu; Kagawa and Tokushima in Shikoku; Hyogo, Osaka, Nara, Shiga and Aichi in Honshu.

Gnathostoma doloresi Tubangui, 1925: In Japan the worm was first found by the author from a pig slaughtered in Tokyo, but, generally speaking, the pig is very rarely infected with the worm. On the contrary, the adult worms are densely parasitic in the gastric wall of the wild boar, *Sus scrofa leucomystax*, almost all over Japan. The first intermediate hosts of the worm are the same as those of *G. spinigerum*. The second intermediate hosts so far found are two species of land salamanders, *Hynobius naevius* and *H. stejnegeri*. In Kumamoto and Miyazaki, Kyushu, they were examined as to the natural infection with the larval gnathostome, and it was found that 30 (8.8 percent) of 342 *naevius* and 1 of 28 *stejnegeri* harboured the larvae. All larvae were found to be encysted in the muscles of the salamanders.

Gnathostoma nipponicum Yamaguti, 1941: At present the Japanese weasel, *Mustela sibirica itatsi*, is the only final host of the worm known. It is of interest that the adult worms are parasitic in the esophageal tumor of the host and not a single worm has been so far found in the gastric wall. The first intermediate hosts were experimentally proved to be the same as those of the other two species, but the second one is still unknown.

VITAMIN E (ALPHA TOCOPHEROL),
A NEW ANTHELMINTHIC AGAINST
ASCARIS LUMBRICOIDES

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The field of anthelmintic therapy has come of age as one most progressive in medicine. But never has there been any report about the use of any vitamin in the treatment of any helminthic infections. Perhaps, some clinicians might have had some similar observations in connection with the administration of this vitamin in the treatment of non-helminthic diseases; but, because they were not parasitologists, they were not able to grasp the significance of them. Or, because this vitamin E has been known only to act catalytically and nutritionally in the human systems, and never has been known to act as an anthelmintic which could have attracted their attention. Or, because the spontaneous expulsion of ascarides is so common among the population that their passing out following the administration of any medication is always interpreted as a mere coincidence. All this is to say that vitamin E has never been thought of to possess any anthelmintic property ever since its discovery in 1922.

However, this report will tell us that there is to this vitamin more than it is commonly known as vitamin E. That it is the real action of this vitamin more than it is ordinarily believed as a mere coincidence. And that the expulsion of the ascarides following the administration of vitamin E can only be due to this vitamin. Obviously, vitamin E becomes now an anthelmintic against *Ascaris lumbricoides*.

METHODS

This came about when a mother with a handful of ascarides in a bottle came to our laboratory to report that the medicine we gave to her two children (8 and 10 years old) had expelled those worms on the fourth day they were taking the same medicine. Our records showed that these two children were having infectious hepatitis, so that only vitamin E (wheat germ oil capsules of 3 mg each) was given, besides the advice that they should not be given any animal fat in their diet. Our dosage of the wheat germ oil to these two cases was 6 capsules a day for each.

Thinking that there could be something in this observation more than met our eyes, we started to pass this idea thru experimental procedures. Since then we had been on the lookout for patients with ascariasis. And since patients of this kind are many in the rural communities, where sanitary level is practically below that of the city, we carried this study in these localities.

In screening patients for this study, we examined the stools of all people coming to our laboratory with complaints of abdominal pain. We ran a number of cover-slip preparations for each specimen; and all those markedly positive (3 plus or 4 plus) were subjected to zinc sulphate floatation method for the determination of the individual's female-ascaride burden. In a matter of a 3-month period, we were able to record 82 cases of ascariasis with mere complaint of abdominal pain, and 8 cases of infectious hepatitis plus the incidental ascariasis.

The 82 cases had ages falling between 7 and 40 years old. We ignored their distributions by sex and occupation because our experience has shown that all individuals are susceptible to ascaris infection. Likewise we did not consider these variables any more as having some importance on our hepatitis cases. They were divided in three age-groups of 7-12, 14-40, and 7-14 of the infectious hepatitis group. This was done for convenience in the dosology of the wheat germ oil capsules of 3 mg each. No parallel control group was carried on in this study, because each subject had his own female-ascaride burden, which served as the standard base line before the treatment. The dosology consisted of 6 capsules of wheat oil (18 mg) in three takes a day for 10 consecutive days for the age-group 7-12, and for the

7-14 of the infectious hepatitis group. The 14-40 age-group was given a dose of 9 wheat germ oil capsules (27 mg) in three takes a day for 10 consecutive days. No other medication was given except the wheat germ oil during the course of treatment of each case even in the hepatitis group.

RESULTS

Like in the hexahydropiperazine hydrate treatment (Hetrazan or Bryrel), the worms came out in variable number from time to time between the second day after the start of the treatment and around three days after the end of the course. The highest number recorded was 6 worms expelled by one bowel movement of one hepatitis case; while in the rest, the worms were expelled at the rate of 1 or 2 per day. The summary results are illustrated in the following table:

Age-group	Number of cases	Total doses per day	Average female ascaride burden	Average number of worms expelled	Efficiency in percentage
7-12. . .	6	18 mg	12	8 (females) 2 (males)	83.33
14-40 . .	22	27 mg	10	6 (females) 1 (male)	70
7-14. . .	8	18 mg	15	13 (females) 2 (males)	100

COMMENTS

For want of a standard technique in determining the intensity of ascaris infection in every individual, a duplicate of Stoll egg counting technique for hookworm was thought of and employed in the stool of every case. The computation was based on 200,000 eggs per female per day. Of course, this is not accurate; but, like any other technique of the same kind, it is for certain an estimate or approximate. Besides, the application of this technique here did not count the number

of males and that of the non-ovigerous females. However, it had served the purpose of a crude base line in the treatment of every case in this study.

The percentage efficiency of the wheat germ oil in every age-group was measured by the average actual number of worms expelled over the basic denominator of the average female-ascaride burden. Again this could not be an actual efficiency because the denominator was composed only of ovigerous females without counting the non-ovigerous females and males. Actually, this might not be correct, but theoretically, the estimated number of females could be taken to reflect the index of infection as well as the basis of efficiency of any drug after the application of the latter. Such being the case, the efficiency ratings here should be taken along this line of thought.

From the viewpoint of understanding the mechanisms of actions of vitamin E on the ascarides, a brush-up on the biochemistry of this vitamin is necessary. In a nutshell, vitamin E or alpha-tocopherol is a fat soluble vitamin, and its rapidity of absorption into the systems is facilitated by the proportionate quantity of the bile present in the intestinal tract. In other words, if there is little bile in the intestines, the absorption of this vitamin becomes impaired towards to an insufficient quantity. The reverse is true if there is plenty of bile. It is absorbed as free tocopherol, and exists in the plasma either free or conjugated with proteins.

However, in the small intestines, by reacting with the phosphates, the unabsorbed tocopherol will become the aqueous soluble phosphate ester of tocopherol. It is this phosphate ester of the vitamin that is highly active at enzymatic level in the small intestines. In the main, its pharmacology is directed towards the inhibitions of many enzymatic oxidative processes. It can inhibit the fatty acid oxidase, peroxidase, proteases, trypsin, phosphorylase, succinic acid oxidase system, hyaluronidase, histamin, and other natural enzymes and anti-enzymes of foreign agents that may be present in the small intestines.

From the preceding pharmacology, we can now see the mode action of this vitamin E on the ascarides. By and large, it is a process of attrition by which the parasites are subjected to. This becomes clear when we realize that the ascarides exist in this intestinal environment because of their anti-enzymes which can neutralize the inimical effects of the intestinal juice which, by itself, consists of so many enzymes.

And also when we realize that the ascarides feed on the absorbable digested nutrients which are by-products of the natural digestive processes. Obviously, the negative states of these enzymatic systems will surely bring about serious effects in various ways on the ascarides.

Take, for instance, the inhibition of the anti-enzymes of the ascarides. If these are contained with or rendered weakened, the ascarides will surely be at the mercy of the injurious effects of the other intestinal enzymes not inhibited by the vitamin E. Likewise, if a great portion of the intestinal juice is inhibited, there will be little food nutrients utilizable by the ascarides. Under this latter condition, the ascarides will get starved; and this together with the inhibitions of their anti-enzymes will surely produce bad effects on the parasites. And when this happens, the only way out for them will be to escape either upwards or downwards of the gastro-intestinal tract. The weaker ones may remain and die. The proper evaluations of these manner of actions can be expressed in terms of the hexylresorcinol and hexahydropiperazine equivalents, which will be the object of our future study.

For the next point, the explanation for a more effective action of the wheat germ oil in patients of the hepatitis group is found in the existence of a bigger quantity of vitamin E in the intestinal lumen as a result of the diminished secretion of bile due to infectious hepatitis. So that in this state of affair, there will be more, and prolonged action of the tocopherol ester phosphate on the ascarides. On the other hand, the lower efficiency observed in the other two age-groups could be due to the smaller amount of vitamin E left unabsorbed as a result of a practically normal amount of bile present in the intestines of every case at the time of treatment.

And for a last comment, the dosology of vitamin E employed in these three age-groups should not be considered strictly. Bigger doses than these may be used to attain more effective results without any fear of any untoward effects of the drug. Because vitamin E is practically harmless even in big doses.

SUMMARY

This is the first time that a vitamin has been found to possess an anthelmintic property. And this is vitamin E (alpha-tocopherol) contained in the wheat germ oil. In the main, its manners of actions are at the enzymatic level. That is, it can

probably inhibit the anti-enzymes of the ascarides; and at the same time, it can inhibit a number of the natural and important intestinal enzymes.

The operations of these two actions in the parasitic habitat of the ascarides can bring about a state of attrition on the parasites — meaning that the parasites get starved, and their resistance to the inimical conditions of the intestinal lumen is reduced very much below their normal threshold level. When this happens, the only way out for the ascarides is to leave this unfavourable environment either actively or passively, or upwards or downwards of the gastrointestinal tract. Its anthelmintic property can be evaluated in terms of the hexylresorcinol and hexahydropiperazine equivalents. This will be the object of our future study.

The preceding explanations were gathered after we had studied its effects on 90 cases of ascariasis divided into three age-groups and under two entirely different conditions. And it appeared from our results that vitamin E was more effective or efficient in the hepatitis group where a high average percentage of expelled worms was recorded. We surmised that this could only be due to a bigger amount of an unabsorbed vitamin E brought about by the diminished bile in the intestines as a result of the infectious hepatitis; and the subsequent prolonged action of the tocopherol phosphate on the ascarides placed under this state of affair.

Finally, this anthelmintic action against *Ascaris lumbricoides* of the vitamin E is a major addition to the long list of its already known actions which are all essential to life. Needless to say, since this is a part of life, and naturally harmless to life within very wide permissible limits, this vitamin E can offer a bid in our search for an ideal anthelmintic against *Ascaris lumbricoides*.

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